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The American Phytopathological Society

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PHYTOPATHOLOGY

VOLUME 25 NUMBER 9

SEPTEMBER, 1935

RESISTANCE OF PINUS SYLVESTRIS TO A GALL-FORMING PERIDERMIUM[1]

W. G. HUTCHINSON[2]

(Accepted for publication December 31, 1934)

INTRODUCTION

In 1926, York (43) reported that a gall-forming Peridermium was causing considerable injury to Scotch pine, *Pinus sylvestris* L., at the Masonic Summer Camp on Round Lake in the vicinity of Woodgate, New York. It has yet not been definitely determined[3] whether this is a new species of Peridermium, some atypical, autoecious form of *Cronartium quercuum* (Berk) Miyabe (*C. cerebrum*), or one of the western-United States gall rusts. Growing among the heavily infected trees in this Woodgate area are many trees that, although artificially inoculated for 6 successive years, have shown no gall formation. The present investigation deals with this phenomenon of resistance as studied from the standpoints of gross morphology, histology, and physiology.

LITERATURE REVIEW

There is a marked paucity of literature, other than observational accounts, on the resistance of forest trees to fungus disease; in fact, there are few recorded attempts to study the basis of resistance in woody plants of any kind. Most discussions relative to the resistance of plants to the invasions of parasites point out that resistance may have a morphological or a physiological basis or a combination of the two. The formation in the host tissue of a definite layer of cork cells in advance of the invading mycelium has been considered by several investigators to represent an attempt to check the spread of the parasite. Conant (9), in studying the cause of resistance of certain strains of tobacco to *Thielavia basicola* (B.

[1] A thesis submitted to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[2] The writer is indebted to Dr. H. H. York for his helpful advice and criticism during the progress of this work.

[3] In the recently published Manual of the Rusts in United States and Canada p. 24, Arthur states "Another rust on *P. sylvestris* . . . recently discovered in northern New York and called 'Woodgate Rust'" is "now referred to *C. quercuum.*"

and Br.) Zopf, recorded that such corky areas between diseased and healthy tissues successfully prevented the spread of the fungus through the plant. Other investigators have stated that the resistant character of cell walls may account for protection against disease. Melander and Craigie (26) found that varieties of Berberis immune from *Puccinia graminis* Pers. were provided with thicker cuticles and with cell walls more resistant to mechanical puncture than were those susceptible to infection. In many instances the products of cell metabolism and their physical and chemical relationships have been considered to have a very direct bearing upon resistance and susceptibility. Dufrénoy (14), from a study of *Blepharospora cambivora* Petri on chestnut, *Phytophthora infestans* (Mont.) de Bary on potato, and *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. on bean, has concluded that the accumulation of phenolic compounds in vacuoles represents a local immunity in the plant. Newton, Lehmann, and Clarke (29) have suggested a relation between the resistance of wheat to *Puccinia graminis tritici* Eriks. and Henn. and the phenolic compounds present in the plants. Cook and his associates (10, 11) have shown that tannin may exert an inhibitory action upon the growth of many species of fungi in culture. A careful biochemical study by Angell, Walker, and Link (4) has recently shown that resistance of onions to onion smudge is definitely related to the presence of protocatechuic acid in the outer pigmented scales. Dickson, Link, and Dickson (12) have found in the case of seedling blight that resistant corn seedlings contained 20 per cent more uronic acids than susceptible seedlings. Ezekiel (16), in an extensive study of resistance of wheat to *Puccinia graminis tritici*, has recorded a correlation between resistance and the growth and branching of germ tubes of the spores in extracts from resistant and susceptible plants. Thomas (36) reports that, although the growth of *Armillaria mellea* (Vahl) Quel. is inhibited by the extractives of the roots of certain trees, there apparently is no relationship to resistance. It is conceivable that the cell sap of certain plants may be uncongenial for fungus growth because of its lack or excess of hydrogen ions. Many experimental attempts have been made to demonstrate such a relationship. Comes (8) has found that a rust-resistant strain of wheat has a greater acidity than susceptible forms. When this same strain, however, is grown under different environmental conditions it becomes susceptible and its cell sap then shows a reduced acidity. On the other hand, many investigators have failed to find any such relationship. Hurd (20), as well as Newton, Lehmann, and Clarke (29), could demonstrate no relation between the pH of cell sap and the resistance of wheat to *Puccinia graminis tritici*. Harris and his coworkers (19) have clearly shown that the osmotic concentration and electrical conductivity of sap expressed from phanerogamic parasites are greater than

those from the host plants. Similar work has been done to determine whether these physical values were any different in resistant and susceptible plants. Newton, Lehmann, and Clarke (29) have been unable to demonstrate any important difference between tissue fluids of wheat plants resistant and susceptible to *Puccinia graminis tritici* as evidenced by osmotic pressure or electrical conductivity.

In addition to the factors considered above, a number of investigators have found that in plants subjected to infection, especially by rusts, there may be a type of reaction referred to as "protoplasmic resistance" or as "hypersensitiveness." This phenomenon was first observed and studied by Ward (37, 38, 39). He noted that in resistant plants hyphae were formed and haustoria penetrated host cells, but that such cells died soon after invasion, and that the hyphae and haustoria degenerated and appeared like experimentally starved mycelium. He attributed this reaction primarily to starvation of an obligate parasite. Marryat (25) studied infection by *Puccinia glumarum* Eriks. & Henn. in both resistant and susceptible strains of wheat. She also considered that resistance was brought about by the death of host tissue resulting in the death of the parasite from starvation. She further suggested that there might be a formation of toxin and antitoxin perhaps mutually destructive. Gibson (18) studied the early growth of various rusts that had infected plants other than their natural hosts. She rejected the starvation hypothesis of Ward, and considered that toxic substances formed by the host cells might be responsible for the failure of the hyphae to form haustoria and for the ultimate death of the mycelium. In natural hosts that were resistant, she found haustoria present, but the mycelium had degenerated after a few days. This phenomenon of hypersensitiveness in wheat infected with rusts also was studied by Stakman (33). He found in susceptible plants a ready adjustment of parasite and host, but an intolerant relationship in resistant plants. The rust hyphae, although forming few haustoria in resistant plants, brought about the death of host cells adjacent to them. They continued to grow for a time and to affect other cells, but soon perished. In some cases haustoria continued to live after the death of the cells that they had penetrated. He, therefore, concluded that the reaction is not due primarily to starvation but to a toxin-antitoxin relationship. Mains (24), investigating infection in strains of corn resistant and susceptible to *Puccinia sorghi* Schw., suggested that the normal symbiosis among cells of the plants is destroyed by the action of the fungus, causing some of them to parasitize others. He considered carbohydrates to play an important rôle in this resistance phenomenon. Allen (1, 2, 3), in a detailed cytological study of wheat resistant to *Puccinia graminis tritici*, has regarded Ward's theory of starvation as "untenable." She has suggested that an antagonistic relationship obtains

in the cell in that the cell is first killed by toxin produced by the haustorium, which in turn is killed by the products of cell degeneration.

The relation of environmental factors to susceptibility and resistance has received much attention. Eckholt (15) is inclined to consider environmental factors, such as the physical and chemical nature of the soil, soil moisture, and soil temperature, of more importance in infection of wheat with *Puccinia glumarum* than hereditary factors of resistance and susceptibility. In an extensive study of wheat grown in nutrient solutions, Doak (13) has recently found that an excess of potassium or lack of nitrogen increases resistance to *Puccinia triticina* Eriks.

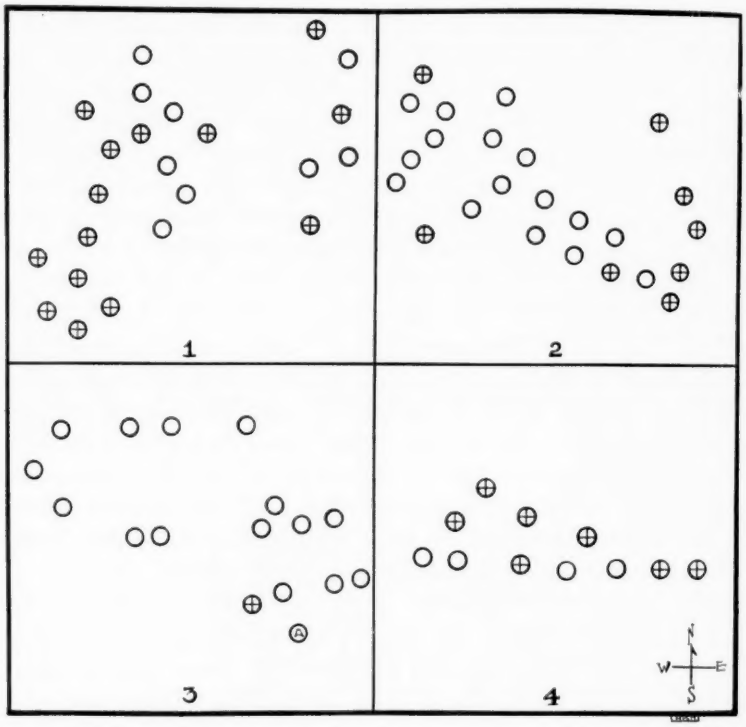


FIG. 1. Diagram of plots of Scotch pine trees. ○, resistant; ⊕, susceptible; A, location of parent tree.

METHODS

Description of plots. In the early summer of 1930, investigations were begun on 4 plots of *Pinus sylvestris* at Woodgate, N. Y. (Fig. 1). Some of the trees in these plots had been inoculated previously by H. H. York. In plot 1, resistant trees of varying age and height are growing near susceptible trees of about the same age and height. Plot 2 consists of

a few trees 8 to 10 years old. About half of these are resistant and half susceptible. In plot 3, there are several trees 10 to 12 years old, all of which are apparently seedlings from one tree, the position of which is indicated by the letter A. This tree, although exposed to natural infection, never developed galls, but succumbed to Armillaria infection. Every tree in this plot except one shows marked resistance. Plot 4 consists of numerous resistant trees about 20 years old growing near groups of susceptible trees.

Inoculations. For 3 consecutive seasons, resistant and susceptible trees have been artificially inoculated. Quantities of galls with mature aecia have been collected in June and masses of aeciospores scraped from them. The terminal portions of branches with the current season's growth were heavily painted with suspensions of these spores in water. The iceless refrigerator described by York[4] was used as a protection about these inoculated branches. This chamber was removed at the end of 48 hours.

Collections have been made from such inoculated branches at intervals varying from 48 hours to 6 years after inoculation. Similar collections have been made from noninoculated trees growing at a distance of 15 miles from the site of the plots, and also a few collections from healthy trees at the Arnold Arboretum, Jamaica Plain, Massachusetts.

Preparation of Tissues. In the preparation of tissues for sectioning several killing and fixing solutions have been used. Formalin alcohol (4 parts formalin to 100 parts 70 per cent alcohol), Carnoy's solution, and Schaffner's chromo-acetic-acid solution have given particular success. Bouin's solution, picro-formol, Fleming's fluid, and Conant's solution also have been used with success.

Some of the tissues were imbedded in nitrocellulose according to the technique described by Jeffrey (21) and Wetmore (41), excellent sections being obtained by this method. In order to obtain serial sections, however, many tissues were imbedded in paraffin by the butyl-alcohol method described by Zirkle (45). This procedure has been found invaluable for the cutting of 1- and 2-year-old stems. Sections, whether cut on the rotary or sliding microtome, were cut from 8 to 15 microns thick. Transverse sections have been used largely, but in many instances radial and tangential sections also.

Staining. The sections were stained with several combination stains. Those intended primarily for the study of normal or abnormal histology usually were stained with the safranin-haematoxylin combination or Fleming's triple stain (6). When it was desired to study the sections for the presence of mycelium and to trace this mycelium through and between cell walls, the combination of orseillin and anilin blue described by Strasburger (35) was used with excellent results.

[4] York, H. H. Celluloid "iceless refrigerator" and some results from field inoculations with the aeciospores of Woodgate rust (*Peridermium* sp.). In manuscript.

Microchemical tests. In connection with the histological study of sections prepared by imbedding, a series of microchemical tests was made on sections freshly cut on the ether-freezing microtome. In most cases the tissues were sectioned a few minutes after they were removed from the tree. Starch, tannin, fats, resin, gums, cellulose, suberin, cutin, lignin, and pectin were identified by means of tests outlined by Eckerson[5] or Stevens (34).

Physiological studies. Quantitative chemical analyses have been made of young twigs from both resistant and susceptible trees with regard to tannin, calcium, and potassium content. Analyses of various samples of soil collected at the base of both resistant and susceptible trees have been made with reference to the quantitative determination of calcium and potassium.

Juices expressed from the twigs of resistant and susceptible trees by pulverizing the ground twigs with liquid air and subjecting this frozen mass to a pressure of 200 pounds per square inch for 10 minutes have been used for determination of osmotic pressure and hydrogen-ion concentration.

An attempt has been made to reproduce in the resistant twigs the pathological changes found following infection. Extractives prepared from galls and from germinating spores were introduced into the tissues through small capillary tubes and also by hypodermic injection. Oxalic acid has been used in the same way, several investigators having reported a relationship of this acid to the pathogenicity of fungi (5, 22).

FIELD OBSERVATIONS OF HOST REACTIONS

In their response to infection the inoculated trees have shown many different reactions ranging from highly susceptible to resistant. For the purposes of this discussion, however, 3 principal types of reaction may be distinguished. Type A occurs only in susceptible trees. During the first growing season there may be slight evidence of infection other than small brownish spots followed by minute swellings. The following spring, however, marked hypertrophies develop and typical galls soon form (Fig. 2, A). In the reaction here designated as type B, there is often definite evidence of infection a few months after inoculation. Isolated discolored areas become confluent and for several inches along the twigs there will be reddish-brown discolorations. During the following growing season an excessive splitting of bark takes place on these twigs, sometimes accompanied by a slight resinosis (Fig. 2, B). Occasionally a few abortive or atypical galls may develop from such areas. In type C reaction numerous small spots may be noted a few weeks after inoculation (Fig. 2, C). These are often greenish brown or slightly reddish in the center, sometimes sur-

[5] Eckerson, S. H. Microchemistry. University of Wisconsin. (Mimeographed).

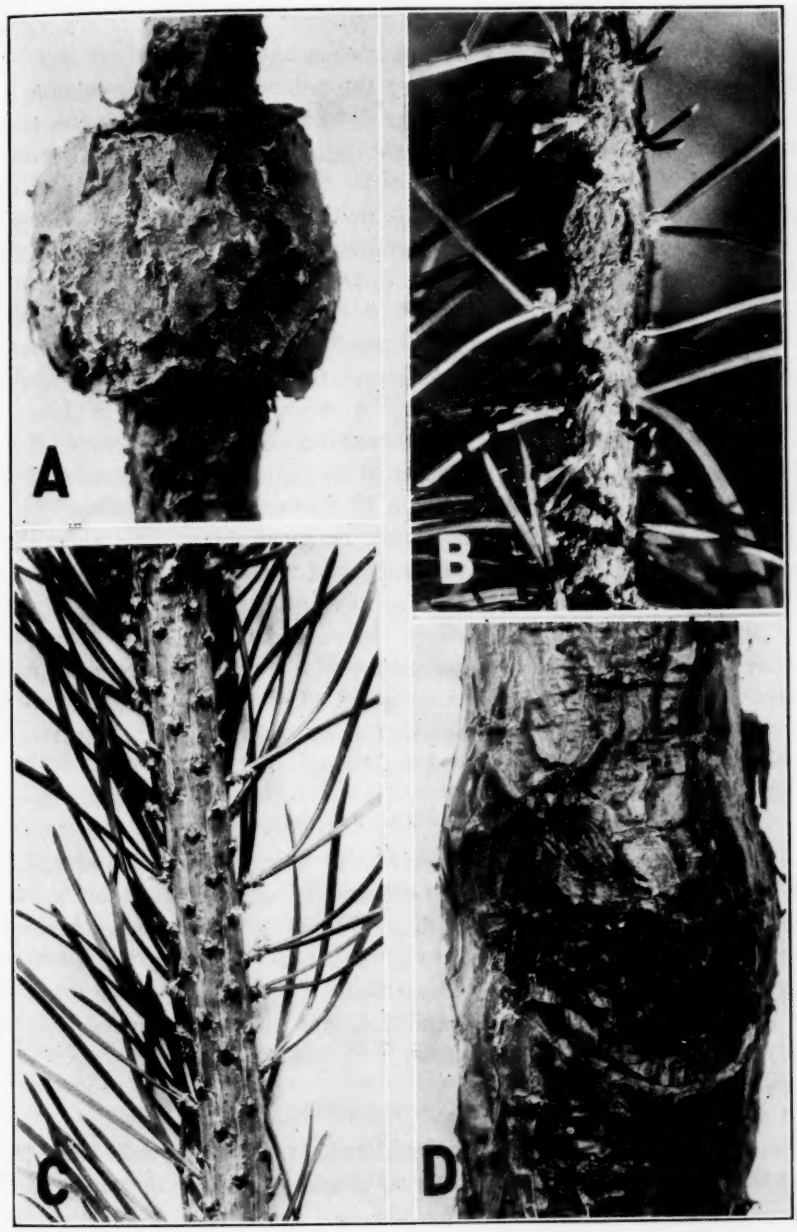


FIG. 2. A. Twig of susceptible Scotch pine 3 years after inoculation showing gall typical of type A reaction. B. Partially resistant twig 1 year after inoculation showing cracked bark characteristic of type B reaction. C. Resistant twig 3 months after inoculation showing spotted areas commonly found in type C reaction. D. Resistant twig 3 years after inoculation showing exfoliation of bark often found in type C reaction. Photographs by H. H. York.

rounded by a lighter chlorotic halo. Similar infection spots have been found developing on various resistant species of pine artificially inoculated with this rust (28, p. 199). During the following growing season, it is not uncommon to find a few swellings developed on these twigs as though galls were to be produced. These swellings rarely increase in size during the third year, but, through the growth of the surrounding tissue, they gradually become indistinct. During the third or fourth year, there can be noted at the position of these swellings a marked exfoliation of bark and sometimes a slight atrophy (Fig. 2, D).

It should be pointed out here that the different reactions described above are not always definitely segregated to certain trees. For instance, types B and C both may be found associated with type A on susceptible trees. Type C, and occasionally type A, may be associated with type B on what we may call partially resistant trees. However, type C only is found on the truly resistant trees. This point will be considered later in connection with the interpretation of the resistant reactions.

Perhaps a brief explanation should be given in connection with the terms "resistant," "partially resistant," and "susceptible," as used above. Gall formation is used as a criterion of susceptibility, since it is only those trees producing galls that show any visible signs of injury. Those trees may be considered partially resistant in which the majority of infection centers are prevented from forming galls. The trees that, although they become infected, are able in some way to check all infection centers and produce no galls can be considered resistant.

HISTOLOGICAL STUDIES

A comparative study of serial sections of noninfected twigs of both susceptible and resistant trees with reference to size of cells and thickness of cell walls has shown no outstanding difference. A microchemical study made of such twigs has shown no significant difference in regard to food storage or chemical character of cell walls. There is considerable variation in the amounts of tannin and fat, but these substances also vary widely among twigs of the same tree.

The Normal Twig

This brief description of the normal twig will include only those structures that have a bearing on penetration and spread of the fungus. The description applies equally to resistant and susceptible twigs during the period when they are subject to infection.

The single layer of epidermal cells is covered over by a cuticle averaging 3 μ in thickness. The cortex consists of 10 to 20 cell layers, the outer 4 or 5 of which rarely store starch during the season when infection might

occur, although the other cell layers may store large quantities. Starch, often in association with fats and resins, also may be stored in the pith, medullary and xylem rays, and phloem parenchyma. Tannin, for the most part, is confined to isolated cells or groups of cells in the cortex, phloem parenchyma, and pith. It also may be found in and near the cambium and in the vicinity of leaf traces. A well-developed periderm may be distinguished during the first growing season. The phellem cells give tests for suberin when tested with ammoniacal gentian violet. A continuous layer of sclerenchyma forms directly internal to the periderm. These cells show a positive lignin reaction. Usually the 3 layers of xylem elements nearest the cambium are free from lignin.

Penetration

The usual method of entrance of the fungus into the host is through the young twigs (44). The germ tube of the spore penetrates the cuticle of susceptible and resistant trees with apparently the same ease (Fig. 3, F and G). No evidence has been secured to show whether the method of penetration, is enzymatic or mechanical.

Growth of Mycelium and Host Reactions

Reaction type A. In a susceptible host, the mycelium is frequently intercellular, forming few or no haustoria for as much as the first month of its development. The cortex cells adjacent to the advancing mycelium become abnormally granular and stain more heavily than normal cells of the same kind. From microchemical tests it can be shown that these cells contain an abundance of tannin, while those more recently surrounded by mycelium are more nearly normal (Fig. 4, B). These latter cells, even at a later stage, do not become so densely granular as do those first attacked. It appears that the cells gradually lose their ability to produce or acquire tannins or else the necessity for tannin formation is withdrawn. It will be noted in figure 4, B, that the haustoria are few and decidedly stunted.

As the mycelium continues to spread through the cortex, the nutritional relationship seems to change, for many large haustoria are formed. The hypha becomes very much constricted as it passes through the minute opening that it produces in a cell wall to form a haustorium (Fig. 3, A). The host cells thus parasitized appear in no way to depart from their normal condition. We have here, then, an example of the almost mutualistic relationship of host and parasite so often reported in the literature, especially in the case of rusts (3, 24, 25, 37, 38, 40).

The early reaction to infection is not always like that just described. Sometimes, in the susceptible host the mycelium forms large haustoria

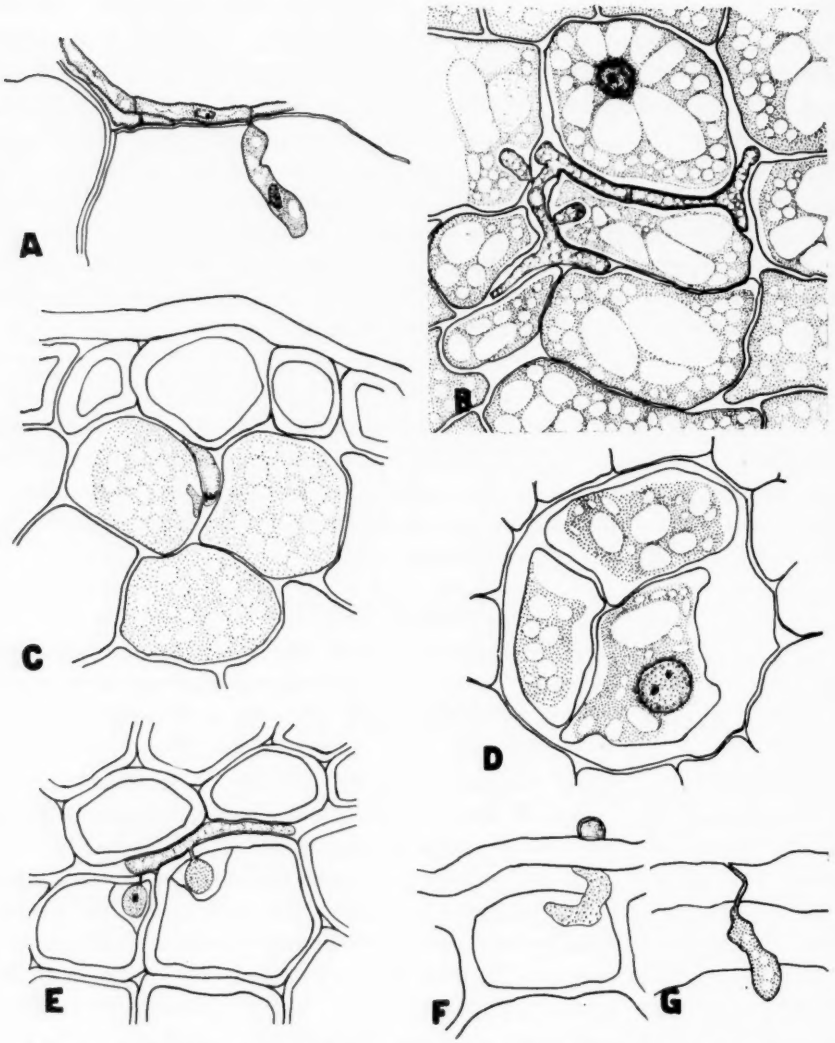
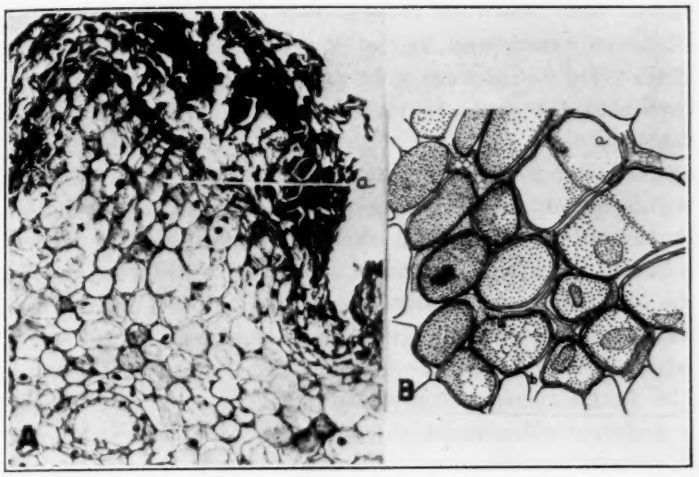


FIG. 3. A. Portion of cortical cell of susceptible host showing mature haustorium. ×1000. B. Portion of cortical region of susceptible host showing intercellular mycelium and penetration of haustoria. ×900. C. Group of epidermal and cortical cells of resistant host showing formation of small lobed haustorium. ×750. D. Giant cell from pathological xylem of resistant stem. ×700. E. Tracheid cells of susceptible host showing haustoria encased in lignified callosities. ×930. F. Epidermal cell of susceptible host showing germ tube which has penetrated the cuticle and is growing over the surface of the epidermal cell. ×950. G. Portion of wall of epidermal cell of resistant host showing the path of penetration of the germ tube through the cuticle. ×1900.



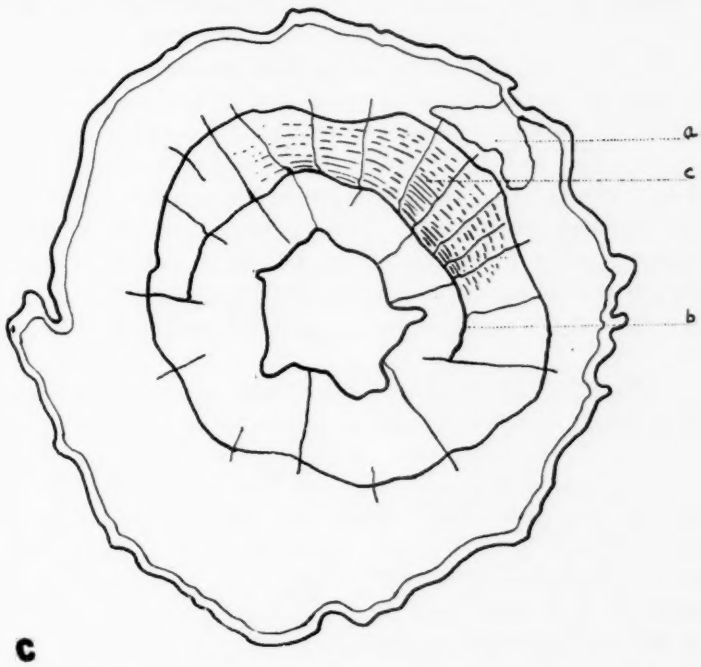


FIG. 4. A. Portion of transverse section of resistant stem of *Pinus sylvestris* one month after inoculation. a. Phellogen below necrotic area showing stimulation. B. Portion of cortical region of susceptible host directly beneath epidermis. The granular cells at the left are those first attacked by the fungus after penetration. These cells contain much tannin. Note stunted haustoria in cells a and b. ×300. C. Diagram of transverse section of resistant stem three months after inoculation. a. Necrotic area in cortex and phloem where cells have died following fungus invasion. b. Pseudo-annual ring formed by inhibited cambium derivatives. c. Region of tangentially elongated tracheids, bending of rays, and excessive formation of secondary rays. ×35.

directly after penetration of the epidermis. The cortical cells do not become filled with tannin and, in fact, appear perfectly normal (Fig. 3, B). In such cases there would seem to be an immediate adjustment of parasite and host without the preliminary hesitation and gradual adjustment as described above.

During the latter part of the growing season in which infection occurred, or the beginning of the following season, this semimutualistic balance becomes somewhat upset in that there is a decided stimulation of the host cells resulting in gall formation. Still, in the developing gall, little change can be noted in recently parasitized cells. A large number of the cells in the developing gall become gorged with starch and may be penetrated by haustoria. Starch is not confined to normal storage tissues, but may be found even in thin-walled tracheid cells. Some cells in the gall have undergone degeneration, and often cells adjacent to these contain much tannin.

It is notable that in reaction type A the mycelium may spread unhindered by any cork barrier. By the end of the season in which infection occurred, the mycelium has advanced to the cambium. Haustoria penetrate many of the newly formed nonlignified xylem elements. As these cells mature the haustoria become surrounded by heavy layers of cell-wall material (Fig. 3, E), forming structures similar to the lignitubers described by Fellows (17); these protuberances usually stain in the same manner as lignified membranes. The haustoria embedded within these protuberances appear to remain alive, but have not been observed to break through this heavy encasement. Much more delicate callosities are apparently formed about haustoria in invaded cortex, phloem, and ray cells.

Reaction Type B. In partially resistant plants the early stage of infection corresponds to that in susceptible plants in that tannin accumulates in cells adjacent to the mycelium. Within a few weeks after inoculation, it can be noted that a group of host cells has died. The cell walls are thickened, and the cells often contain brownish or yellowish globules. Mycelium can rarely be seen within or between these dead cells, but is abundant in adjoining areas, where it forms many haustoria. A suberized layer, usually 3 to 4 cells wide, is formed enclosing this necrotic area, as well as the infected tissue adjacent to it. The mycelium advances to this layer of wound cork and in many cases breaks through it, infecting the healthy tissue beyond. As a result, some of these infected cells die, a second cork layer may be formed, and the progress of the mycelium again checked. Three such alternating layers of necrotic tissue and cork have been observed in some twigs. When the mycelium finally gains a foothold and is no longer checked, a gall may be developed, but it is often

aborted or atypical. Usually, however, the mycelium seems to degenerate, becoming unable to break through the second or third cork barrier, and finally disintegrates. As the twig continues to grow, the bark is split and there is an exfoliation of the necrotic areas. This may continue for 2 or 3 seasons and may sometimes be accompanied by a generalized swelling in this area. This would appear to be due to the stimulation of the cambium in a manner probably similar to that described under reaction C.

Reaction type C. Sections through the discolored infection spots commonly found on all trees after artificial or natural inoculation, but frequently the only symptom of infection in the resistant trees, show small necrotic areas in which often no mycelium is evident. As soon after penetration as the fungus comes in contact with the subepidermal cells, these cells respond by becoming intensely granular with tannin, as described above. They also develop thickened cell walls, at least on the side near the mycelium. An occasional haustorium (Fig. 3, C) may penetrate these cells before this response occurs. As the mycelium makes feeble progress, the cortex cells first attacked continue to develop even thicker walls, and the cells, instead of remaining granular, become filled with large globules that, in unstained sections, appear yellowish brown. Microchemical tests would indicate that some of these globules are composed of gum, and others of oil or fat, both types being in some way associated with tannin. Haustoria in such collapsed cells occasionally retain nuclei and appear normal for a short time before their death and disintegration.

Sections examined one month after inoculation, at a time when these infection spots are visible macroscopically, show definite necrotic areas, often extending as far as the phellogen (Fig. 4, A). Many cells are collapsed, the majority contain the globules described above, the cell walls are thick and often lignified, and the entire area takes a very heavy stain with safranin. The cells adjacent to such areas show a stimulation in that the nucleus is increased in size and the cells show an increased staining ability. In the nearby phellogen regions, tangential division of the cells has occurred to a greater extent than in the normal portions of this tissue. A search for mycelium or haustoria among this group of dead cells has been uniformly unsuccessful. These necrotic areas, then, may be considered similar to those reported in various resistant hosts infected with rusts (3, 24, 25, 33, 40). These regions of necrotic tissue are soon sloughed off as the twig continues to develop.

In these resistant twigs, as mentioned above, an occasional swelling may form, as though galls were to be produced. In the development of such areas, it would appear that there is a delayed response in the first-attacked cortex cells. The formation of tannins, the thickening of cell walls, and

finally the development of globules and the collapse of the cells do not accompany the early infection. Consequently some of the hyphae may progress to other normal cells before they are checked by the death of the first-invaded tissue. In this way the mycelium is able to reach the cambium during the first growing season. The hyphae are by no means abundant, but tend to be fragmentary and can be seen only with difficulty. During the progress of the parasite, the host cells directly back of and adjacent to the advancing hyphae have died, and there is consequently formed a wedge-shape area of necrotic tissue extending from the epidermis to the cambium (Fig. 4, C). The majority of the cells in this area are dead. Globular contents of cells and excessively thickened cell walls are evident. Some cells that have not died possess very large nuclei and may contain much starch or oil. This wedge-shape area is separated from the normal cortex and phloem by a layer of wound cork. Adjacent to this the cells show the result of stimulation in that they stain much more heavily than similar normal cells, and are often gorged with starch. Early in the growing season following that in which infection occurred, a layer of sclerenchyma is formed adjacent to the necrotic area and directly external to the wound cork. Any mycelium that may be distinguishable at this time appears to be dead and disintegrating.

Cambial development during the latter part of the first season results in the formation of extremely pathological tissue. A portion of the cambium, often embracing half of its circumference around the twig, may be almost completely inhibited in development (Fig. 4, C), while the rest of the cambium is developing normally. The few divisions that may occur in these inhibited cells yield derivatives that fail to mature but appear to collapse and die very soon after formation. Later in the same growing season, however, this inhibited tissue may become greatly stimulated. Individual cambial cells increase in size, especially in a tangential direction, often becoming 4 to 6 times as long as normal cells. The tracheids, as well as phloem elements derived from such cambium cells, are also very much elongated tangentially. The stimulus that brings about this pathological condition often continues to act during the second season after inoculation, although it appears to be greatly weakened, for the cambium and its derivatives gradually become normal.

The xylem elements, formed just prior to and during the attack of the fungus on the cambium, some of which may be invaded by haustoria, do not complete their differentiation, but in many cases remain thin walled, retain cytoplasm and nuclei, and may be excessively pitted. Quantities of starch and oil may be stored in such juvenile xylem cells. Giant cells are frequently produced (Fig. 3, D) and it is not uncommon to find the

formation of tylosis-like cells extending through a pit in one tracheid into another. According to Chrysler (7) this phenomenon in pine is a result of wounding. Resin ducts in such areas are filled with tylosis-like cells in which starch may be stored. Rays developing through such abnormal areas are composed of abnormally large cells in which also much starch and oil may be stored. There is a very noticeable bending of these rays (Fig. 4, C), the change in direction of growth occurring at the point where the cambium is inhibited and its derivatives killed.

The study of sections through infected areas, cut during the latter part of the third year after inoculation, usually shows that the stem tissues have recovered from the effect of the stimulus. The juvenile tracheids and the layer of dead-inhibited-cambium derivatives in the first-year xylem compose what appears like a pseudo-annual ring extending half way or more around the stem (Fig. 4, C). External to this may be a considerable area of tangentially elongated or abnormally large tracheids. The tracheids of the late second-season and entire third-season growth have become gradually shorter tangentially, and those nearest the cambium are entirely normal. The bending of the rays at the position of the pseudo-annual ring is evident throughout the abnormal portion of the stem. An unusually large number of secondary rays arise as the tissues become more nearly normal (Fig. 4, C). The cambium as well as the more recently developed phloem tissues appear normal. The wedge-shape necrotic area has become completely surrounded by wound cork and sclerenchyma. The entire area stains heavily with safranin, and many of the thickened cell walls appear to be lignified. During the next growing season, this area is gradually crowded out nearer and nearer to the exterior of the twig. Portions of it are gradually sloughed off and finally the whole area is thus removed. The study of the progress of these later stages is often complicated by the fact that there may be a secondary infection, possibly by a wood-rotting fungus.

From the standpoint of pathological anatomy, the juvenile tracheids that retain cytoplasm and nuclei and are checked in their differentiation typify a hypoplasia (23). On the other hand, the formation of enlarged tracheids, giants cells, and tyloses in tracheids and resin ducts would represent a hyperplastic condition. The entire area of pathological xylem can be considered as wound wood.

In view of the large amount of pathological tissue formed in these stems, there might be some doubt about referring to them as resistant. However, since the fungus is definitely checked in these tissues, never comes to fruition, and furthermore does not sufficiently stimulate the tissues to produce a gall, these stems may well be considered resistant.

PHYSIOLOGICAL STUDIES

Chemical Analyses

Collections of twigs of the current season's growth were made from 12 resistant trees varying in age from 8 to 15 years. Each tree had been artificially inoculated for several successive years, but had failed to produce any galls, the reaction to infection being always of type C. These trees were typical of the entire group under consideration, as they were chosen from the different plots, some growing in partial shade and others in full sunlight, some growing on hillsides and others on level ground. Collections also were made from 12 susceptible trees, 8 to 15 years old and growing under conditions similar to those described above. Each tree had numerous mature galls. These collections from susceptible trees were further divided into one group consisting of twigs without galls and into another group containing only galls.

The selection of samples for analysis was made by dividing each twig into several portions and making a random choice of these parts after they had been thoroughly mixed.

Analyses have been made particularly for tannin,[6] potassium, and calcium.[7] The results expressed in per cent by weight are as follows:

	Resistant	Susceptible	Galls
Moisture-free basis			
Soluble solids	12.9	13.8	16.3
Non-tannins	8.7	9.9	11.2
Tannin	4.2	3.9	5.1
As plant ash			
CO_2 and C free ash	1.2	0.93	
CaO	29.86	33.32	
K_2O	41.40	18.09	

Perhaps the two points of significance in these results are the great excess of potassium in the resistant plants and the higher content in the galls of both tannin and other soluble solids. It is recognized that these results should not be given undue weight, as they represent but one determination. Yet the striking difference in potassium content of these two carefully chosen samples may be of considerable significance. The rôle of potassium in the plant (27) is thought to be closely connected with the synthesis of carbohydrates and probably of proteins and oils also. If,

[6] The tannin analysis was made by the Division of Industrial Farm Products, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

[7] The potassium and calcium analysis was made by Mr. H. J. Hallowell, Consulting Chemist, Philadelphia, Pa.

then, resistance may have some nutritional basis, this potassium relationship may be fundamentally related to it. It has been repeatedly shown (13, 30, 31, 32), that plants grown with a lack of potassium are more susceptible to disease than those grown with sufficient potassium.

Analyses of samples of soil from both surface and subsoil[8] collected from the base of three resistant trees and three susceptible trees growing under the same conditions in very close proximity have failed to reveal any significant difference in potassium or calcium content. The following results are based on air-dried soil:

Trees	Sample	Potassium %	Calcium %
Resistant	Surface soil	1.24	0.60
	Subsoil	2.29	0.80
	Surface soil	2.20	0.60
	Subsoil	2.29	0.73
	Surface soil	2.29	0.52
	Subsoil	2.49	0.47
Susceptible	Surface soil	2.65	0.63
	Subsoil	2.53	0.94
	Surface soil	2.61	0.58
	Subsoil	2.69	0.74
	Surface soil	2.37	0.62
	Subsoil	2.52	0.68

Relation of Cell Sap to Growth of the Fungus

In a further attempt to note some physiological difference between susceptible and resistant trees, a study was made of the effect on the fungus of juices extracted as described above from noninfected susceptible and resistant trees. Since it has not been found possible to produce mycelium by culturing rusts on any sort of culture medium, the criterion of fungus growth could be only spore germination and a comparatively slight growth of the germ tube. The spores were sown in drops of the liquids on depression slides and kept at room temperature. In view of the fact that tannin has been found in some cases to be a deterrent to fungus growth, the effect of various strengths of tannin dissolved in distilled water has been similarly studied.

The results of these studies are summarized in table 1. It will be noted that the juices from susceptible and resistant trees have approximately the same relationship to the growth of the fungus. The resistant juice does not show the deterring effect that might be expected, but even would

[8] The soil analyses have been made by the Division of Soil Fertility, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

appear to increase the percentage of germination. The results of the study with tannin indicate conclusions similar to those reached by Cook and Taubenhaus (10), *i.e.*, that low percentages of tannin solution may stimulate fungus growth, perhaps acting as food, but that higher percentages may inhibit or prevent growth.

Physico-chemical Constants of Cell Sap

Samples of juices freshly expressed from resistant and susceptible twigs as described above were used to determine osmotic pressure by means of the Beckman thermometer, and hydrogen-ion concentration by means of the potentiometer. The following constants have been secured:

Sample	pH	Osmotic pressure in atmospheres
Resistant	3.84	13.60
Susceptible	3.76	13.69

The rather close correspondence of these figures would indicate very little difference between the resistant and susceptible trees in these respects.

Injection Experiments

The formation of abnormal tissues in the resistant twigs, even after the mycelium had been checked and had disintegrated, would indicate that the stimulation may be due to some chemical formed by the disintegrating mycelium or host cells. Two sets of injection experiments have been performed to attempt to induce such abnormal changes by chemical means. An extract was made of mature galls by grinding them with sand and distilled water and filtering the liquid through a Seitz filter. A portion of the filtrate was shaken with aluminum hydroxide and refiltered, much of its brownish color being removed by this process. Another extract was prepared by germinating spores until the germ tubes had reached their maximum length. They were then frozen in distilled water so that the germ tubes were ruptured and the liquid was then filtered.

These three extracts were introduced into the plant tissues, both by hypodermic and capillary injection.

In the same manner 1 per cent and 0.1 per cent solutions of oxalic acid were injected into twigs. Controls of injections of distilled water and simple needle punctures were made on several trees.

During the course of 3 months some of the injected twigs developed slight swellings and resinosis. Collections were made at intervals of 1 month for 3 months, and the tissues prepared and stained as described

TABLE 1.—*Germination and growth of germ tube of spores of Peridermium sp. sown in various dilutions of tannin and in juices extracted from susceptible and resistant trees*

Medium	No. of spores examined	No. of spores germinated after 4 hours	Germination per cent	Average length of 10 longest germ tubes after 4 hours μ	Average length of 10 longest germ tubes after 18 hours μ
Juice extracted from uninfected resistant trees	1135	88	7.8	57	192
Juice extracted from uninfected susceptible trees	1143	32	2.8	68	192.5
Tannic acid— 1%	780	0	.....	.....	.....
" " 0.1%	316	0	.....	.....	.....
" " .075%	1060	185	17.4	105	.....
" " .05%	565	53	9.4	88.2	.....
" " .025%	1200	77	6.4	74.2	.....
" " .01%	900	40	4.4	30.0	225
Distilled water	360	0	.....	.....	.....
Tap water	800	20	2.5	83.8	210

above. A comparative study of the control twigs and those injected with chemicals has revealed no cell or tissue abnormality other than could be attributed to wounding. Adjacent to the wound caused by the needle or tube several cell layers had collapsed and died. Internal to this area a cicatrice layer had formed about the entire wound. For several cell layers internal to this there was a decided stimulation of cells in that they stained more heavily than similar normal cells and the nuclei were often larger than in normal cells. There appeared to be no stimulation of the cambium due to the diffusion of the extracts or acid into this tissue.

DISCUSSION

Studies of resistance among plants to parasitic organisms have revealed the fact that this resistance may be morphological, physiological, or functional in nature. It has further been shown that the resistance of a plant may depend, not on a single phase, but may perhaps be determined by all phases.

In the present study attempts have been made to correlate resistance with some morphological feature or with some physiological aspect of the

plant and, in a study of the reactions of the plants to inoculation, to note any differences that might help to explain resistance.

Twigs from noninfected resistant and susceptible trees have shown no histological or microchemical differences that might account for the different reactions to invasion by the rust. In relation to the physiological aspect, the only outstanding difference noted is the potassium content of the noninfected twigs. Perhaps too much significance should not be placed upon this fact. Yet, in view of the generally accepted rôle of potassium in the plant as a catalyst in carbohydrate formation, the relationship of the excess of potassium in resistant trees to their resistance might well be investigated further.

It is, however, from a study of the reactions of the plants to inoculation that most can be learned in this case about the nature of resistance. The fungus in one plant may progress unimpeded and establish itself in an almost mutualistic relationship with the host, while in the tissues of another plant, or in the tissues of another portion of the same plant, the host cells may be killed almost immediately and the fungus partially or completely checked in its progress. The cause of these markedly different host reactions is not easily determined.

It is well first to consider how the results of this study may be correlated with the starvation hypothesis suggested by Ward (37). The complete checking of the parasite by the collapse and death of invaded host tissue might be considered to be the result of the inability of the fungus to secure food from its living host cells, and its further inability to adapt itself to any saprophytic mode of life.

The occasional spread of the mycelium from such necrotic areas, especially when the host reaction has been a mild or gradual one, would appear to depend upon the vigor of the fungus. This vigor is without doubt determined by the amount of food the hyphae are able to secure before the necrosis sets in. This food may be transferred to the advancing hyphal tips, which are thus able to grow beyond the dead tissue areas. If the fungus in its food absorption can keep ahead of the host necrosis, it may be able to advance even to the cambium during the first season. If, however, the host necrosis becomes more rapid, the mycelium may lose its vigor and degenerate since it is surrounded by dead host tissue. In the susceptible host the cells would appear to be stimulated to acquire additional food material for the use of the fungus. The fungus thus increases in vigor and advances unhampered through the tissues.

True[9] has noted that even in the most susceptible Scotch pine trees very heavy inoculations with this same species of Peridermium may result in no

[9] True, R. P. Gall development on *Pinus sylvestris* attacked by the Woodgate Peridermium and morphology of the parasite. In manuscript.

gall formation, but in the cracking of bark characteristic of reaction type B. This fact might also be explained on a nutritional basis. The numerous mycelia spreading from several infection centers may defeat each other in their struggle to secure food from the host and none may be able to establish itself.

The same explanation may be given for the failure of many infection centers to develop in the susceptible host, although from one such center the mycelium spreads and produces a gall. The mycelium that is able to establish an immediate congenial relationship with the host cells would appear to secure almost a monopoly on the food supply.

As Wellensiek (40) has pointed out, starvation may result not only from the entire lack of individual food substances, but may be brought about by only a slight variation in the quantity of some food material. The apparent difference in potassium content between suceptible and resistant twigs may indicate some carbohydrate excess or deficiency, as suggested by Mains (24).

The failure to demonstrate by injection experiments the presence of any toxic material in the fungus should not invalidate an assumption that toxin production may take place in the living cell invaded by haustoria. Allen (1) has considered that such an assumption may best account for resistance in wheat. There is evidence that the death of host cells precedes the death of the invading haustoria; similar evidence has been obtained by Allen (1) and Stakman (33). It would thus appear possible that a toxic substance released by the fungus is responsible for the death of the host cells. The disintegrating cell contents in turn may release materials harmful to the haustorium. It also is conceivable that in these poisoned host cells the food available for the fungus may be rendered unavailable, and the haustorium will soon die from starvation. On the other hand, in reaction type A the toxin produces no visible injury, but stimulates the cells to increase in size and number. It would thus appear that cells showing the resistant reaction are hypersensitive to the excretion of the fungus.

It has been noted that a layer of cork cells develops about the infected tissue in both reaction types B and C. This is later followed by the formation of a continuous layer of sclerenchyma cells about this region. This corky layer might at first be considered to have some definite relation to resistance. However, the same type of tissue may be formed as a reaction to mechanical wounding. Invaded tissues that respond by collapsing and becoming necrotic are in reality wounded tissues and function as a pseudo-cicatrice layer (42). The continuous layer of cork may then be considered a cicatrice layer and thus to represent solely a response to wounding. The usual lack of cork development in those infected areas in the susceptible host from which galls develop is apparently due to the fact that here the

host cells are not injured or wounded. Only when the fungus is low in vigor and apparently unable to secure sufficient nourishment is it checked by this cork layer. Thus this layer should be considered only meagerly related to resistance.

The accumulation of tannin in those cortical cells first attacked by the fungus has been found accompanied by at least a partial checking of the mycelium. While tannin also has been observed in abundance in tissues of wounded control twigs, it would seem to have some bearing upon resistance in this case. As has been pointed out, the mycelium may spread rapidly in the susceptible host without any accumulation of tannin in the host cells. In other susceptible twigs the mycelium makes slow progress until it grows beyond the tannin-containing tissues. In some resistant, as well as some susceptible twigs, the mycelium makes no progress beyond the tissues in which tannin accumulates. It is quite probable that other factors besides tannin accumulation are involved in this partial or complete retarding of the fungus. It is not clear in just what manner tannin may help to bring about this reaction. Haustoria growing into such tannin-filled cells are not usually killed until the cell itself has died. The study of spore germination and growth of germ-tubes in various percentages of tannin might indicate that the action upon the parasite is somewhat toxic.

This accumulation of tannin in host cells might well be considered as representing the "local immunity" suggested by Dufrénoy (14) for similar accumulations of phenolic compounds.

SUMMARY

A comparative study of stem tissues and extractives of *Pinus sylvestris* trees susceptible and resistant to a species of Peridermium has been made in order to determine the nature of resistance to this parasitic fungus.

No correlation has been found between any morphological feature of the noninfected host and the resistance to infection.

Three general types of reaction to inoculation may be distinguished: (1) typical gall formation on the susceptible host; (2) cracking of bark and slight resinosis sometimes followed by the formation of atypical galls on the partially resistant host; (3) formation of small necrotic areas on twigs followed sometimes by swellings that do not increase in size after the first or second year but are gradually sloughed off.

In the most susceptible plants the mycelium may actively grow in the host and live in something of a mutualistic relationship. This results in stimulation of living cells and the formation of a gall.

In the most resistant trees the host cells are killed immediately after invasion. The fungus also soon dies and the necrotic area is sloughed off.

Often, in both resistant and susceptible plants, the first-invaded cells

may become filled with tannin. This reaction may represent a local immunity.

In those resistant trees that develop swellings, mycelium grows to the cambium and then dies. Areas of necrotic tissue extend from epidermis to cambium. The cambium becomes stimulated to form quantities of wound wood including tangentially enlarged tracheids, giant cells, and tyloses in tracheids and resin ducts.

In the resistant reaction, the necrotic area becomes surrounded by cork and later by sclerenchyma. This probably represents a normal reaction to the wounding of cells, and may play a small part in resistance.

No correlation has been observed between the osmotic pressure or hydrogen-ion concentration of cell sap and resistance.

A greater amount of potassium has been found in noninfected resistant stems than in susceptible stems. This is thought to have some possible relationship to resistance on the basis of nutrition.

It is considered that the liberation of toxin into cells by the haustoria may cause the death of the hypersensitive cells of the resistant host. The fungus is thus deprived of its food supply and soon dies. In the susceptible host, however, toxin does not kill the cells at once but simply stimulates them to gall formation.

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GRAFT VERSUS INSECT TRANSMISSIONS OF CURLY TOP IN TOMATOES (TOMATO YELLOWS)

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PROCEDURE

The curly-top virus, causing a disease in tomatoes known as tomato yellows (4), was previously transmitted by the writer through approach grafts from diseased to healthy tomato plants (6). The technique employed in these early experiments also was used in subsequent trials. In addition to very young seedlings, older plants were used both in the greenhouse and in the field. Slices about 1 inch long and a depth of $\frac{1}{4}$ to $\frac{1}{3}$ of the diameter of the stem were cut off from the opposite sides of each plant, the exposed surfaces were brought in contact and the two plants were tied firmly together. Because of its flexibility, the electrician's rubber splicing compound was found very well suited for the purpose and caused no injury to the plants. The approach graft, when completed, appeared as shown in figure 1. Only in certain exceptional cases did growing plants grafted in this manner fail to unite.

One of the plants of the graft pair was exposed to viruliferous beet leaf hoppers, *Eutettix tenellus* Baker, either before or after grafting, as required by the experiment, and the effect of this exposure on both plants was studied. As a rule, 10 insects were used to each plant, confined in a celluloid cell tied over a leaf or a tip of the plant (5). The insects were removed after about 5 days of feeding, and the tape was cut away about a week or 10 days after grafting. If at that time a union was formed the cion of the insect-inoculated plant was severed from the stock just below the graft region.

EXPERIMENTS

The original purpose of grafting work in connection with tomato yellows was to find a simple and efficient method of transmitting this disease to take the place of the customary slow and cumbersome procedure of the beet leafhopper transmission. Had grafting proved to be such a method, it would have been expected that the diseased tomato cions would provide the inoculum. Accordingly, the earlier trials dealt with grafting small diseased shoots or buds on healthy tomato plants. These experiments resulted in almost complete failure, as only an occasional bud would form the union and

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FIG. 1. A pair of tomato plants united by means of an approach graft for the purpose of transmitting curly-top virus from an insect-inoculated plant to a healthy plant.

only one out of 21 plants so grafted contracted the disease. The diseased cions and buds had very little if any vitality or power for regeneration. No further attempts were made to graft diseased cions on healthy plants.

Since, however, it was observed that often plants show a tendency to regenerate (2), it was of interest to ascertain whether whole diseased plants may not possess greater ability to form successful graft unions with healthy plants than small branches of these plants. This led to the subsequent experiments with approach grafting. Such a method of inoculation could not, however, be looked upon as a proper substitute for the usual procedure of insect transmission, as it required the latter to begin with and then the graft in addition, but it gave other interesting data for the study of the virus passage between a pair of graft-united plants.

The approach grafts at the very outset proved to be a much better means of obtaining a perfect union of the grafted plants as well as of transmitting the disease to healthy uninoculated companions of the graft pair. However, when plants showing fully developed curly-top symptoms were used for grafting, the percentage of failure to form a union with healthy plants was still too high and the transmission practically nil. Table 1 shows that in the group of 13 grafted pairs, of which one plant showed well-developed symptoms of yellows, only 1 healthy plant contracted the disease out of 8 successful grafts.

TABLE 1. *Results of grafting diseased and healthy plants. Stages of tomato yellows: S—slight, leaves roll or purple, but no yellowing; M—medium, leaves begin to turn yellow and show more rolling and vein purpling; Y—severe stage, very little of green color left. PU—perfect union; No U—no union. Grafts were made in a greenhouse at Berkeley, California, on December 22, 1930. Results recorded on January 6, 1931*

Conditions of diseased plants at grafting	Final results
S	PU, no transmission, diseased plant dead
S	PU, no transmission, diseased plant recovered
M	PU, disease transmitted, diseased plant dying
Y	PU, no transmission, diseased plant dead
Y	"
Y	"
Y	"
Y	"
Y	No U, no transmission, diseased plant dead
Y	"
Y	"
Y	"
Y	"

It was decided, therefore, not to wait for the development of symptoms, but to graft the plant exposed to viruliferous insects with a healthy noninoculated plant, either simultaneously with the exposure to insects or shortly afterwards. For the sake of brevity, the individuals on which the insects were fed are called inoculated plants, whereas those grafted to these inoculated plants are called grafted plants. In all counts given in the following tables the pairs that formed no union are excluded.

As shown by a series of 15 tests, with the number of good unions varying in individual tests from 12 to 98, a greater number of plants invariably became diseased on the inoculated side than on the grafted side. Apparently, in some cases, the virus present in the inoculated plant found some unsurmountable obstacle in crossing the graft bridge connecting the diseased and

the healthy plants. The exact differences in these 15 tests are given in table 2.

TABLE 2.—*Showing curly-top transmission through approach grafts in tomatoes*

Group	Total No. of pairs grafted	No. diseased plants in		Differences between (a) and (b)
		(a) Inoc. group	(b) Corresponding grafted group	
I	19	11	8	3
II	12	9	7	2
III	15	10	9	1
IV	23	21	11	10
V	24	24	7	17
VI	22	21	5	16
VII	24	23	11	12
IX	48	46	11	35
X	44	40	37	3
XI	28	20	16	4
XII	28	22	6	16
32/2	95	87	77	10
32/3	98	94	78	16
33/A	15	8	7	1
33/B	15	3	2	1
Total	510	439	292	147

It should be noted that, so far, in no case did the disease appear in any plant of the (b) group without showing in the corresponding plant of the (a) group.

That the unfailingly smaller number of affected plants in the group of individuals grafted with insect-inoculated plants, as compared with the latter, is significant may be seen more clearly from table 3 where actual figures are changed to percentages of the total number of inoculated pairs forming perfect unions. In the preparation of tables 3 and 5 to 8 inclusive, Love's modification of Student's method of statistical evaluation (3) has been followed for its simplicity and adaptability to small numbers in paired experiments. The Z values were calculated from the formula:

$$m \div \sqrt{\frac{\Sigma}{n}(d-m)^2}$$

in which m is the mean of differences between a and b (with d representing individual differences between a and b) and n, which denotes the number of trials or samples. In accordance with the Z and n values, odds were taken from Love's table. Groups a and b in table 3, 7, and 8, while not independent variables, form natural pairs with reference to the virus movement from one plant to the other and from cion to stock.

TABLE 3.—*Indication of relative significance of slack in the curly-top virus transmission in tomatoes through the graft*

Group symbol	Total No. of pairs grafted	Percentage infected of the total grafted		Differences		
		In the insect inoc. (a)	In the healthy grafted (b)	a–b (d)	d–m	$(d-m)^2$
		Per cent	*Per cent*			
I	19	57.1	42.0	15.1	−15.6	243.36
II	12	72.2	61.1	11.1	−19.6	384.16
III	15	66.0	58.9	7.1	−23.6	556.96
IV	23	92.6	44.9	47.7	17.0	289.00
V	25	100.0	31.3	68.7	38.0	1440.00
VI	22	95.0	21.7	73.3	42.6	1814.76
VII	24	95.9	45.8	50.1	18.4	338.56
IX	48	95.9	22.9	73.0	42.3	1789.29
X	44	87.7	82.5	5.2	−25.5	650.25
XI	28	70.2	55.8	14.4	−16.3	265.69
XII	28	77.3	22.7	54.6	23.9	571.21
32/2	95	91.6	81.0	10.6	−20.1	404.01
32/3	98	95.9	79.6	16.3	−14.4	207.36
33/A	15	53.3	46.6	6.7	−24.0	576.00
33/B	15	20.0	13.2	6.8	−23.9	571.21
				460.7		10101.82

With the data presented in the above table $Z=\frac{30.7}{25.9}=1.2$, and odds approximately 3332 : 1 against the occurrence of differences as great as those obtained in this experiment being due to chance alone. This being the case, it was logical to assume that if several plants (instead of two) were grafted together in chain-like series, the plants farthest removed from the insect-inoculated plant would show a smaller percentage of the disease than the plant directly grafted to the inoculated one. To ascertain such a possibility, a field experiment was conducted in which 4 plants in each hill were grafted as illustrated by the diagram in figure 2. The results of this experiment (Table 4) show a tendency toward a gradual decrease both in severity and in percentage of the disease. The severity in this case was judged by the number of dead plants.

It is apparent, even from a superficial examination of tables 1 and 2, that there is a considerable variation between differences obtained in the amount of the disease on two sides of the graft union. A closer analysis of the circumstances under which these grafts were made indicated that a certain correlation existed between the time elapsed from the moment of inoculation to the moment of graft and the degree of success of the graft

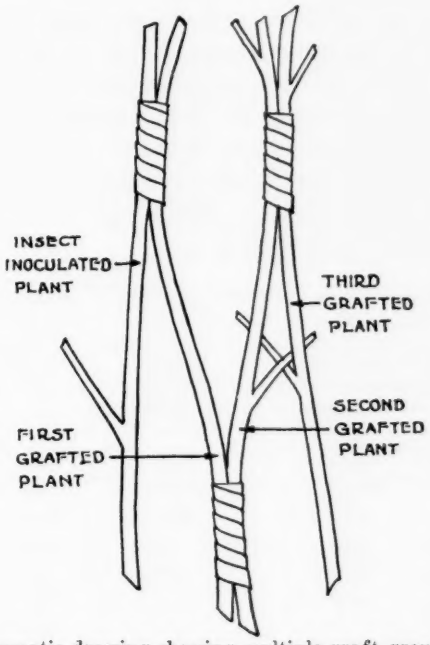

FIG. 2. A diagrammatic drawing showing multiple-graft group used in the curly-top transmission studies.

transmission of the disease. In accordance with this observation all grafts were grouped into 3 classes as follows: (I) advanced grafting group, comprising those pairs that were grafted several (usually 5 or 6) days prior to the inoculation of one member of the pair; (II) simultaneous grafting group, in which inoculation of one member immediately followed the grafting operation; and (III) delayed grafting group, comprising those pairs of which one member was inoculated several (usually 5 or 6) days prior to its

TABLE 4.—*The development of curly top in tomatoes with quadruple grafts*

Number of affected and dead plants	Inoc. plant	1st grafted plant	2nd grafted plant	3rd grafted plant
Row 1 (15 hills inoc.):				
Total No. of affected plants	14	13	11	10
Total No. of dead plants	11	8	6	6
Row 2 (16 hills inoc.):				
Total No. of affected plants	16	10	10	9
Total No. of dead plants	15	5	3	2

being grafted to a healthy plant. Correspondingly, tables 5 and 6 give the relative results of these experiments and show that the best transmission of the curly-top virus through the approach-graft union was obtained when the inoculation of one member of the pair was made almost simultaneously with its grafting to the other healthy member. No advantage was secured from an advanced grafting; there may have been even a slight disadvantage. Significant differences developed with a delayed grafting, giving, in this case, the poorest disease transmission.

TABLE 5.—*Simultaneous vs. advanced grafting of diseased with healthy tomato plants (percentage of infection)*

Group symbols	Grafted simultaneously with inoc. (a)	Grafted 5–6 days in advance of inoc. (b)	Differences		
			a–b (d)	d–m	$(d-m)^2$
1932 1	94.4	84.4	10.0	5.4	29.16
2	80.9	75.0	5.9	1.3	1.69
3	73.7	85.0	−11.3	−15.9	252.81
4	70.0	76.2	− 6.2	−10.8	116.64
5	80.0	84.4	− 4.4	− 9.0	81.00
1933–	46.6	13.2	33.4	28.7	823.69
			27.4		1304.99

Accordingly, $Z = \frac{4.6}{14.75} = .31$ and odds = 2.85 : 1 against the above differences being due to chance, as they probably are.

TABLE 6.—*Immediate vs. delayed grafting of diseased with healthy tomato plants (percentage of infection) in development of curly top*

Group symbols	Time of grafting after the removal of insects		Differences		
	24 hrs. or less (a)	2 days or more (b)	a–b (d)	d–m	$(d-m)^2$
	Per cent	*Per cent*			
I. III	90.0	72.7	17.3	− 8.9	79.21
IV, V	66.0	29.2	36.8	10.6	112.36
VI, VII	47.8	23.8	24.0	− 2.2	4.84
IXa, IXb	45.5	4.3	41.2	15.0	225.00
Xa, Xb	87.7	77.3	10.4	−15.8	249.64
XIa, XIb	60.0	33.3	26.7	0.5	0.25
XIIa, XIIb	36.4	9.1	27.3	1.1	1.21
			183.7		672.51

In this case $Z = \frac{26.2}{9.8} = 2.67$ and odds = 2499 : 1 in favor of the above differences being significant.

As stated in the introductory chapter of this article, the tape uniting 2 grafted plants was removed 7–10 days after grafting and, if a good union was evident, the grafted inoculated plant was cut below the graft junction and the insect-inoculated cion was left to grow on the originally noninoculated plant. The final results as to the transmission of the disease were based on the symptoms developing in the cion and the plant to which it was attached. The appearance of the disease symptoms in the cion, however, was not always accompanied by their development in the parent stock from which it was severed, and *vice versa*. In this connection it was deemed of interest to ascertain whether the cion or the stock was resistant to the successful development of the virus. The data presented in table 7 show that, although there is a slight preponderance in favor of cions, the difference does not seem to be unquestionably significant.

TABLE 7.—*Percentage of curly-top infection in cions vs. severed stocks (bases) of tomato plants*

Group	Cions (a)	Bases (b)	a–b (d)	d–m	$(d-m)^2$
	Per cent	*Per cent*			
A–1 (19)	89.5	84.4	5.1	– 1.4	1.96
2 (16)	93.7	62.5	31.2	24.7	610.09
3 (20)	90.0	95.0	– 5.0	–11.5	132.25
4 (21)	95.2	80.9	14.3	7.8	60.84
5 (19)	89.5	89.5	0.0	– 6.5	42.25
B–1 (18)	94.4	100.0	– 5.6	–12.1	146.21
2 (21)	95.2	85.7	9.5	3.0	9.00
3 (19)	100.0	89.5	10.5	4.0	16.00
4 (20)	95.0	80.0	15.0	8.5	72.25
5 (20)	95.0	100.0	– 5.0	–11.5	132.25
1933 (30)	36.6	40.0	– 3.4	– 9.9	98.11
			66.6		1321.11

With $Z = \frac{6.5}{10.95} = .59$, odds approximately = 22 : 1 indicating that the differences obtained in this experiment might well be solely due to chance.

There was, on the other hand, a decidedly significant difference in the number of recoveries from the disease, the bases or stocks of inoculated plants showing this tendency to a much greater degree than the cions, as may be seen from table 8.

This gives $Z = \frac{13.0}{9.3} = 1.4$ and odds = 908 : 1, decidedly against the occurrence of the above differences as being due to chance alone.

TABLE 8.—*Percentage of recoveries in severed bases vs. cions of tomato plants*

Group	Bases (a)	Cions (b)	a–b (d)	d–m	$(d-m)^2$
A–1	5.3	5.3	0.0	−13.0	169.00
2	12.5	0.0	12.5	− 0.5	0.25
3	15.0	0.0	15.0	2.0	4.00
4	14.3	0.0	14.3	1.3	1.69
5	36.6	10.5	26.1	13.1	171.61
B–1	0.0	0.0	0.0	−13.0	169.00
2	4.5	4.8	− 0.3	−13.3	176.89
3	22.2	0.0	22.0	9.0	81.00
4	25.0	5.0	20.0	7.0	49.00
5	20.0	0.0	20.0	7.0	49.00
			129.0		871.44

DISCUSSION AND CONCLUSIONS

It is obvious that grafting in the curly-top work with tomatoes can not take the place of the standard insect-inoculation method if simplicity and promptness are desired. For the best graft transmission it is necessary for the diseased plants to be in a very early stage of the disease, preferably before the appearance of distinct symptoms, which means a preliminary insect inoculation. Even with these requirements fully satisfied a certain variable number of plants fail to obtain the virus from the infected cions with which they form a union. The reason for this failure is not definitely known although a suggestion may be seen in the character of the cell structure in the graft region. As shown by Dufrénoy and Shapovalov (1), even successful graft unions contain a barrier of degenerated cells between the living tissues of the two plants, which are only partially connected by bridges of hyperplastic cells. Therefore, it may be surmised that the passage of virus from one plant to another under such conditions is made difficult and sometimes, perhaps, impossible.

It is more difficult to understand the failure of the curly-top symptoms to appear in the stock when the inoculated cion served after its union with a healthy plant develops these symptoms and *vice versa*. If the size of the plant is to be considered a factor, then both, apparently, should have had even chances for overcoming the spread of the infectious principle, the cion as being a part of another healthy plant and the stock as comprising nearly all of the original inoculated yet vigorous individual (Table 7). This does not, however, explain why the stocks showed a greater ability to recover from the disease than cions after an early appearance of the symptoms (Table 8).

The following conclusions may be drawn from the experiments described in this paper:

1. The infection of one of a pair of grafted tomato plants with curly-top virus does not assure the passage of this virus to the second grafted plant.

2. This failure of the second grafted plant to contract the disease from the other (infected) plant through the graft union occurs even when grafting and inoculations are made simultaneously, or when grafting precedes the inoculation and it is much greater when grafting is delayed.

3. The insect-inoculated shoot (or cion) may fail not only to transmit the disease to the healthy plant with which it is grafted, but may recover entirely from all symptoms of the disease after being severed from its mother plant (or base) below the graft union, or may never develop the symptoms, regardless of the condition of the mother plant from which it was severed.

4. The base, when severed from a diseased cion as above, may likewise fail to show any curly-top symptoms or it may recover from the disease, although the cion may continue to develop these symptoms transmitting the infection to the healthy plant with which it is grafted.

5. The number of recoveries among the bases of the inoculated plants was decidedly greater than among the cions severed from them.

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THE INFLUENCE OF PHYTOMONAS TUMEFACIENS AND PHYTOMONAS RHIZOGENES ON THE ACTUAL ACIDITY OF CERTAIN LIQUID AND AGAR SUBSTRATA[1]

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INTRODUCTION

The physiological interrelations of the host plant and the bacteria of crown gall and hairy root present many complex problems requiring separate detailed study. One of these problems, the change induced by the growth of the organisms in the pH value of the bacterial colony and of the substratum, has formed the basis of the present studies.

Previous work on this subject has been confined to changes in the pH value in liquid cultures after a lapse of a set period of time (7), (1), (8), (12), (6), (9), (4). As far as could be ascertained, little if any work has been done either on the sequence of changes induced by the growth of these organisms, or on the changes induced on agar media. Indications that differences might exist between the pH value of an agar medium and that of the bacterial colony growing upon it were afforded by Hendrickson *et al.* (4). These authors found that if aniline blue were added to a yeast-infusion manitol mineral-salts agar medium, adjusted to the neutral point of the indicator, the colonies of the crown-gall organism assumed a blue coloration, while the substratum remained colorless. This raised the question whether the phenomenon was brought about by differences in pH value, absorption, or some other factor.

The incubation temperature appeared worth consideration because of the influence of temperature on the pathogenicity of the crown-gall organism. Riker (7) found that, whereas both host and parasite grow well above and below a critical maximum temperature range of 28°–30° C., galls were induced by inoculations on tomato kept below but not above this temperature range. For this reason it was considered advisable to make a study of the pH relations of *Phytomonas tumefaciens* at 26° and 32° C. Parallel with this experiment a similar one was carried on with a nonpathogenic culture of the crown-gall organism (4). It appeared likely that such studies would

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indicate whether or not pathogenicity was correlated with the pH-temperature relations.

MATERIALS AND METHODS

Cultures of the crown-gall organism designated A-6 and A-6-6 were employed throughout the investigation. A-6 is the progeny of a single-cell isolation made by Wright *et al.* (12) and is pathogenic. A-6-6 is the progeny of an isolation made from A-6 by Hendrickson *et al.* (4) and is nonpathogenic. The culture of the hairy-root organism used was designated C-10 and is the progeny of a single-cell isolation also made by Wright *et al.* (12). The cultures were tested for pathogenicity both before and after the experiment. In both tests A-6 and C-10 proved pathogenic, while A-6-6 proved nonpathogenic. On the completion of each series of experiments, the viability of the bacteria was demonstrated by the growth of subcultures taken from both liquid and agar cultures.

Carrot-infusion and yeast-infusion glucose mineral-salts media were employed throughout the experiment. The carrot-infusion medium was prepared by steaming 200 g. of minced carrot in one liter of distilled water for 1 hour. The pH value of this medium was not adjusted. The composition of the yeast-infusion glucose mineral-salts medium[3] was as follows: 1 per cent yeast-infusion, 100 cm.^{3}; calcium chloride, ($CaCl_2$), 0.1 g.; sodium chloride, (NaCl), 0.2 g.; magnesium sulphate, ($MgSO_4 \cdot 7H_2O$), 0.2 g.; dipotassium phosphate, ($K_2HPO_4 \cdot 3H_2O$), 0.2 g.; glucose, 5 g., and distilled water, 900 cm^3. In later experiments the amount of the dipotassium phosphate was increased to 0.5 g., thus obviating difficulties experienced from the drop in pH value during autoclaving. The pH value of the medium was usually in the region of 6.8 and no adjustment was made. Agar media were prepared by adding 2 per cent shredded agar to the liquid media.

The cultures employed for transfers to the experimental series were made on yeast-infusion slants. These were kept at room temperature (21° to 22° C.) and were used when 4 days old. Aqueous suspensions of the organisms were prepared by adding 10 cm^3. of sterile water to each of the slants.

The tube cultures used in the experiments contained 10 cm^3. of liquid medium, the plates approximately 25 cm^3. of agar medium. In sowing the liquid tube cultures, 2 drops of the aqueous suspension were placed in each tube by means of a sterile pipette. The agar plate cultures were sown with 2 streaks approximately 5 cm. apart, made by lightly passing a sterile cotton-wool swab, moistened with the aqueous suspension of the organism, over the surface of the medium. Cultures were incubated at 26° and 32° C. and in order to prevent evaporation the plate cultures were placed in round metal containers, each holding 30 plates. Occasional fluctuations in temperature occurred, which, however, never exceeded 1° C.

[3] Hereinafter referred to as yeast-infusion medium.

The pH values were determined in preliminary experiments with the aid of glass electrodes. The quinhydrone equipment also was employed, as described later. The circuit utilised in conjunction with the glass electrode was of the simple potentiometric type, with the inclusion of a vacuum tube, as described by De Eds (3). The glass electrode was of the membrane type, having an internal diameter of approximately 4 mm. This electrode was prepared after the manner described by MacInnes and Dole (5) and was used in combination with a saturated calomel half-cell. Calibration was carried out each time, both before and after use, against a series of standard buffer solutions. The method employed in taking the readings was as follows: the electrode assembly, comprising the electrode and a potassium chloride-agar bridge, was fastened to the horizontal arm of a dissecting microscope, a delicate control thus being maintained by means of the rack screw. The electrode assembly was then lowered either into the liquid medium or on the surface of the bacterial growth. With the agar cultures, 2 readings were taken on each of the 2 streaks, the end of the electrode assembly being washed with distilled water after each reading. Difficulties experienced from induced currents set up by the movements of the operator, were lessened by the use of a grounded copper-gauze screen surrounding the electrode.

An attempt was made to run a parallel series of determinations of the oxidation-reduction intensities of the cultures. The taking of readings on the liquid cultures proved simple, but all attempts to obtain reproducible readings on the bacterial growth on agar media failed. In consequence, further reference to this aspect of the problem has been omitted.

Quinhydrone electrode readings were taken on the same cultures in parallel with the glass electrode readings. The method employed in taking the readings on the bacterial growth on the plate cultures was as follows: the bacterial growth was removed from the surface of the medium with cotton-wool swabs and placed in small glass tubes, where it was diluted with approximately 3 cm^3. of carbon dioxide-free distilled water. Even greater dilution was found to have no effect on the value of the readings.

Often considerable variation was found in the glass electrode readings on different parts of the streaks, owing presumably to an unequal rate of growth, but by taking the pH value of the whole bacterial growth, with the quinhydrone electrode, an average was obtained that was considered preferable.

A comparison of 56 readings taken on a series of cultures with both the glass and quinhydrone electrodes showed that, on an average, readings taken by means of the quinhydrone electrode on the growth on agar media were 0.32 of a pH unit lower than those obtained by means of the glass electrode. Readings taken with both electrodes on liquid media were almost identical.

A comparison of curves drawn from data secured by means of both electrodes showed that they were closely related in form. Since these comparisons demonstrated that the quinhydrone electrode could be employed for the work on agar cultures without loss of accuracy, it was used exclusively in later studies.

In continuation of the work of Hendrickson (4) with the indicator medium, readings were taken on the agar medium both below and midway between the streaks of growth on the plate cultures, in order to determine whether any considerable difference existed between the pH value of the bacterial colony and that of the substratum upon which it was growing. The samples of agar were removed by means of a cork borer and were ground in a small glass tube into a paste with a suitable amount of quinhydrone, to which was added approximately 3 cm^3. of carbon dioxide-free distilled water. As with the bacterial growth, even greater dilution was found to have no effect on the value of the readings. With the liquid cultures, a sample was decanted and mixed with quinhydrone in the usual manner. All samples were agitated until a constant reading was obtained.

RESULTS

Four series of determinations were carried out, involving a total of approximately 1,400 cultures and 4,000 readings, including duplication on each cultures.

No greater differences were noted between results at the two temperatures than those that might be ascribed to the slower growth of the organisms at the higher temperature. For this reason, in order to economise space, the graphs drawn from data obtained at 32° C. have been omitted. The graphs of data obtained from one representative series at 26° C. are given in figure 1.

The pathogenic (A-6) and nonpathogenic (A-6-6) cultures of the crown-gall organism, growing on yeast-infusion liquid medium, although not parallel in action, did not lower the pH value of the medium beyond what might be expected from absorption of carbon dioxide, nor did they show much drop in the pH value of the bacterial mass on the agar medium. These observations are in accord with the report by Riker *et al.* (8) that this organism does not produce acid from glucose.

A slightly greater fall in pH value was brought about by the growth, on agar medium, of the nonpathogenic strain of the crown-gall organism (A-6-6), than by the pathogenic strain (A-6).

The sharp initial fall in the pH values of the agar control in the series presented in figure 1 was not of constant occurrence. In other series the agar controls varied from having no initial drop to this extreme.

In cultures of the hairy-root organism (C-10), which produces acid from glucose (8), it was seen that, whereas in the liquid medium there was a

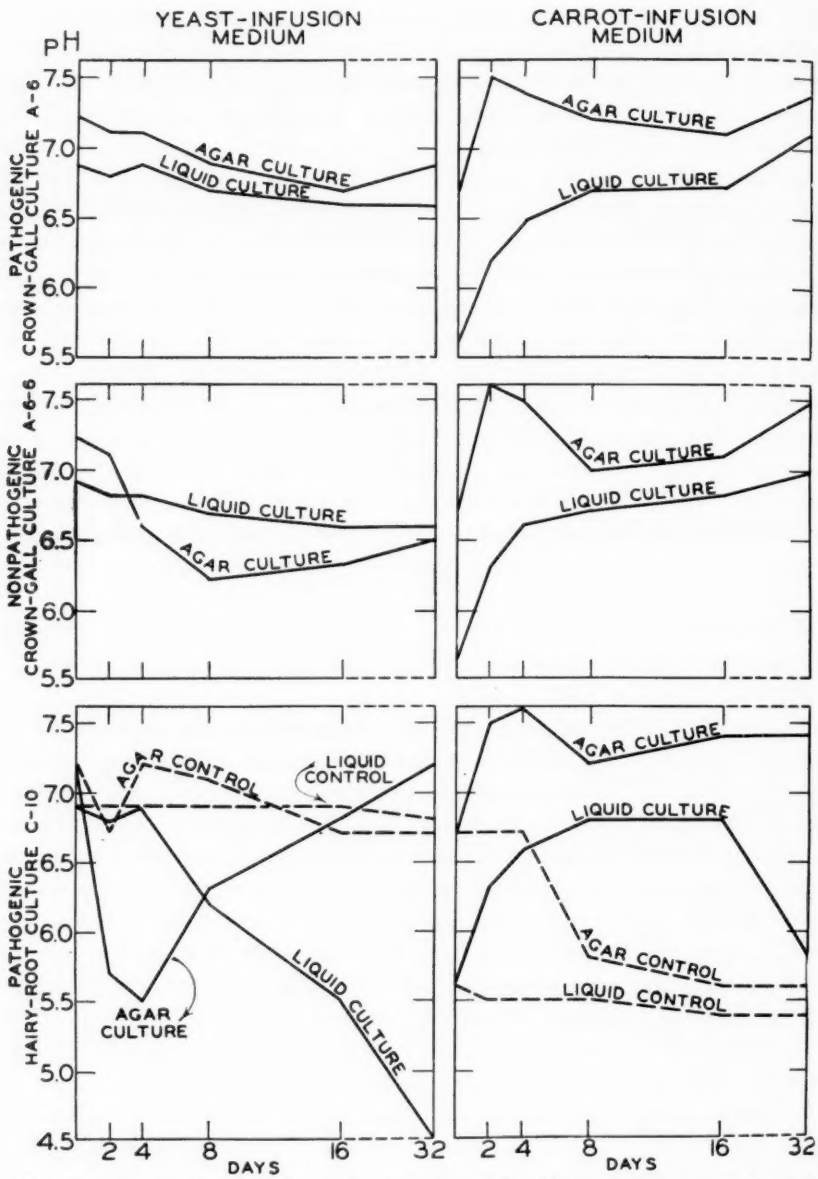

FIG. 1. Changes in pH value in both agar and liquid carrot-infusion and yeast-infusion glucose mineral-salts media, induced by the growth at 26° C. of pathogenic and nonpathogenic strains of the crown-gall organism and by that of a pathogenic strain of the hairy-root organism.

gradual fall in pH value, on agar media there was a very rapid fall followed by an almost equally rapid rise.

Readings on cultures on carrot-infusion media also showed differences in pH values induced in liquid and agar media. In liquid medium there was a gradual rise in pH value, often followed by a considerable fall in the cultures of the hairy-root organism. On agar medium there was a rapid rise in pH value in the cultures of all 3 organisms, followed by a slight drop towards the acid side. This latter phase was not constant in the cultures of the hairy-root organism, although it is shown to have occurred in the series illustrated in figure 1.

The data obtained on the changes in pH value induced in the agar substratum appear to bear out what might be expected from diffusion. In figure 2 is shown a typical graph drawn from data obtained in the manner

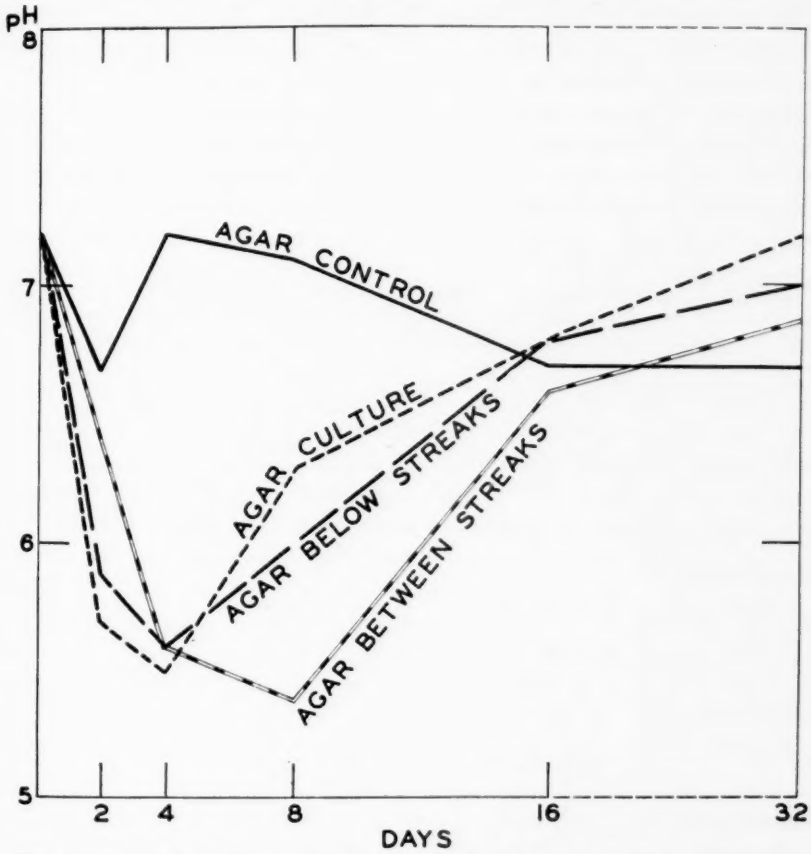

FIG. 2. The relation between the pH value induced in the bacterial growth and that induced in the agar substratum, below and midway between the streaks of growth. The graph illustrates data obtained from readings made on a culture of the hairy-root organism, growing at 26° C. on yeast-infusion glucose mineral-salts agar.

previously described. The pH value of the medium below the bacterial growth approximated that of the growth itself, though in most cases it showed a certain amount of lag. The pH value of the agar midway between the streaks showed a more marked lag.

In one of the preliminary series of readings, it was found that the control plates showed a marked rise in pH value instead of the normal gradual fall occasioned by the absorption of carbon dioxide. Qualitative tests with Nessler's reagent proved that this rise was caused by the production of large amounts of free ammonia by the plate cultures of the hairy-root organism. This free ammonia was produced in sufficient quantity to affect control plates stored in a separate metal container in the same incubator. In all series following this discovery, the hairy-root cultures were stored in a separate incubator.

The method employed in testing for ammonia did not permit the Nessler's reagent to come in contact with the medium. One drop of the reagent was placed on a microscope slide that was then laid on the surface of the baterial growth in the agar cultures. With the liquid cultures a similar slide was placed in an empty Petri dish into which was decanted a portion of the tube culture round the sides of the slide. In both cases readings were taken after the lapse of half an hour. The results of this investigation are presented in table 1. Adequate controls were maintained that showed both

TABLE 1.—*The production of free ammonia by a nonpathogenic and by a pathogenic strain of the crown-gall organism, and by the hairy-root organism, at 26° C. on liquid and agar carrot-infusion and yeast-infusion glucose mineral-salts media*

Media employed	Age of culture	Ammonia in culture employed[a]		
		Crown gall		Hairy-root
		Pathogenic	Non-pathogenic	Pathogenic
	Days			
Carrot-infusion liquid	2, 4, 8 and 16	+	+	+
" " "	32	0	0	+
Carrot-infusion agar	2, 4, 8, 16 and 32	0	0	+
Yeast-infusion etc. liquid	2	0	0	0
" " "	4, 8 and 16	0	0	+
" " "	32	0	0	0
Yeast-infusion etc. agar	2, 4, 8 and 16	0	0	+
" " "	32	0	0	0

[a] The presence of ammonia is indicated by +; its absence, by 0.

the sterile media and the air of the laboratory free from ammonia. From a study of the data it is seen that in both liquid and agar yeast-infusion and carrot-infusion media, the hairy-root organism produces free ammonia. In similar experiments with the crown-gall organism, no positive reaction to the test for ammonia was obtained, except in the carrot-infusion liquid medium. At the 32-day period, in those instances where free ammonia had previously been liberated, there was either a diminution in the strength of the reaction with the testing reagent, or else the test yielded a negative result.

DISCUSSION

Conner *et al.* (2) have shown that on yeast-infusion agar containing 1 per cent glucose, the hairy-root organism ferments most of the glucose within the first 10 days of growth; while in liquid medium, where the growth is not so rapid, the fermentation of the glucose is not completed in 30 days. They explain the absence of acids in the solid medium after the early period of growth by assuming that the pyruvic and acetic acids, formed by the fermentation of the glucose, have been either broken down or neutralized. These facts would account for the rapid fall followed by a rise in pH value, noted during the present work, in cultures of the hairy-root organism on yeast-infusion agar.

The slightly lower pH value induced by the growth of the non-pathogenic strain (A–6–6) of the crown-gall organism on yeast-infusion agar, while of doubtful significance in relation to pathogenicity, should be borne in mind as the only difference, revealed by this investigation, between the pathogenic and nonpathogenic strains. The results secured in these studies were similar at both 26° and 32° C., which temperatures are, respectively, below and above the critical maximum temperature range for gall formation (7). The temperature data secured, in addition to the slight difference noted between the pathogenic and nonpathogenic strains of the crown-gall organism, give no indication that pathogenicity is governed by the pH value induced.

The use of agar media in the present investigation has demonstrated that considerable variation in results may be obtained through the use of approximately similar media having different physical characteristics. This emphasises the dangers of comparing artificial and natural substrata.

The production of ammonia has an intimate bearing not only on changes in pH value, but also on the nitrogen metabolism involving the interrelationship of the host and parasite. Smith, Brown and McCulloch (11), Smith (10), Riker (7) and Riker *et al.* (8) reported the production of ammonia from liquid media by *Phytomonas tumefaciens*. In most of these cases the media contained peptone, which served as a source of ammonia

during fermentation. Riker *et al.* (8) also reported that *P. rhizogenes* produced ammonia from a peptone medium.

The rapid rise in pH value in cultures on carrot-infusion medium is, in all probability, caused by the formation of ammonia during the process of protein degradation. Several explanations of the subsequent fall in pH value, where such occurs, are suggested, including: (1) reduced ammonia production coupled with the formation of carbon dioxide or other acid substances from the medium; (2) utilization of the ammonia by the crown-gall organism. Sagen *et al.* demonstrated that of the crown-gall and hairy-root organisms, only the former is capable of utilising the ammonium radicle as a sole source of nitrogen. This fact, coupled with the data presented in this paper, shows clearly that a distinct difference exists between the nitrogen metabolisms of these two organisms.

SUMMARY

The changes in pH value induced by the hairy-root and by both pathogenic and nonpathogenic crown-gall bacteria on agar media were more rapid than those found in corresponding liquid media.

In yeast-infusion glucose mineral-salts agar medium, little change in reaction was brought about by the crown-gall organism, whereas the hairy-root bacterium produced a rapid fall in pH value followed by an almost equally rapid rise. In the corresponding liquid medium the growth was slower, resulting in a gradual fall in pH value throughout the 32-day period of the experiment. In carrot-infusion medium a rise in pH value occurred in cultures of all three organisms.

The nonpathogenic strain of the crown-gall organism differed from the pathogenic culture only by producing slightly more acid in the yeast-infusion glucose mineral-salts agar medium. No appreciable difference in pH value was noticed between cultures of the pathogenic and nonpathogenic strains of the crown-gall organism when they were grown, respectively, above and below the critical maximum temperature for gall formation.

It was found that the pH value of agar substrata, measured below and midway between the streaks of growth, followed that of the bacterial growth, with a lag that depended on the distance from the bacterial growth.

Free ammonia was formed on liquid and agar carrot-infusion and yeast-infusion glucose mineral-salts media by the hairy-root organism, and on liquid carrot-infusion medium alone by the crown-gall organism.

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EFFECT OF CERTAIN CHEMICALS ON THE "COMBINATION STREAK" VIRUS OF TOMATOES

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INTRODUCTION

Comparatively little has been done on the effect of chemicals on plant viruses. Plant pathologists lacked a satisfactory incentive for this type of research, as it seemed to promise little in the way of results that would be applicable to the control of virus diseases or very helpful in differentiating various viruses unless these could readily be obtained in a highly purified form. The most extensive work of this kind is that of Allard (1, 2), who tried a considerable number of substances on the tobacco-mosaic virus (presumably Johnson's virus no. 1). Johnson (14), himself, conducted some chemical trials with this virus and several other viruses causing various forms of tobacco mosaic, cucumber mosaic, spot necrosis, ring spot, and tomato stem necrosis. Fukushi (13), Lojkin and Vinson (19) and Caldwell (3) worked with tobacco mosaic and Doolittle (9) with cucumber mosaic. In these experiments over 80 substances were tested on the green tobacco mosaic, 8 on cucumber mosaic and only 1 or a few on each of the remainder.

However, with the exception of spot-necrosis experiments all the above-mentioned work dealt with simple viruses only. The field of virus complexes, particularly the possibility of their splitting into simpler component parts, was left practically untouched. The writer has taken up a study of the reaction of the so-called tomato "combination streak" virus to some of the most promising chemicals as far as the inactivation of either the entire complex or any of its portions is concerned. These studies have not been concluded, but as there is no prospect of their immediate resumption it is deemed justifiable to publish the results thus far obtained, as they may prove interesting and useful to other workers.

By "combination streak" is meant a naturally occurring complex virosis of tomatoes, which may be split into two component parts: (A) Green tobacco mosaic (Johnson's virus no. 1) and (B) latent potato mosaic, found in nearly all commercial stocks of American varieties of potatoes, but absent from healthy seedlings. It is thus designated in order to distinguish it from other forms of tomato streak, such as the dieback form of the Pacific Coast (apparently identical with English and Australian "spotted wilt"), experimental and glasshouse streaks described by English workers, two

[1] The writer feels greatly indebted to Dr. T. E. Rawlins, of the University of California, Berkeley, Calif., for his most helpful advice and suggestions as well as a quantity of valuable materials received from him in the course of this work.

simple streaks reported by Doolittle and Blood (10), and a number of unnamed forms now being studied by various investigators.

"Combination streak" becomes evident through necrotic lesions of various types on leaves, petioles, and stems, such as spots, streaks, and irregular blotches. It is accompanied by mosaic and crinkling of the leaves. When the leaf surface is mostly covered by these lesions the petiole is girdled, the affected leaves die, but acronecrosis, as a rule, is absent and the diseased plant continues to grow, although from the production viewpoint it is worthless. The usual method of isolating the component parts of the complex consists in the use of differential hosts, some of which are susceptible only to one form of mosaic. For the sake of brevity the combination form of streak will be referred to hereafter merely as streak, the green tobacco mosaic as mosaic A, and the latent mosaic as mosaic B. This use of the letters is identical with that employed by Samson.[2]

METHODS EMPLOYED

Fresh tomato plant material from plants affected with combination streak was crushed in a clean, dry food chopper and the juice was strained from the pulp through a piece of cheese cloth. No filtration or purification was attempted. The chemicals chosen for testing were first dissolved in distilled water. These solutions, as well as other liquid chemicals, were added to the strained juices to make up the desired concentrations. The mixtures were allowed to stand 2 hours before application to test plants. The control portions of the juice were mixed with the corresponding amounts of distilled water and also held for 2 hours before application. During this time all mixtures were shaken several times to insure homogeneity.

The inoculation tests were nearly all carried on under greenhouse conditions. Small tomato seedlings, averaging 5 to 6 inches in height, were used. At this age they are extremely susceptible to this form of streak. Check plants, inoculated with nontreated juices from tomato plants affected with combination streak, showed 100 per cent infection in each case and will be omitted from inclusion in the tables. All juices were brushed on the leaves with a camel-hair brush or a cotton swab. As a rule, symptoms of either streak or mosaic appeared 4 or 5 days later. It required 10 days or 2 weeks, however, for the symptoms to develop on some of the plants. If no symptoms appeared within 30 days, both viruses are considered permanently inactivated, or if one of the two mosaics failed to appear that virus was regarded as inactivated.

In selecting the chemicals for this work the principal attention was

[2] Samson, R. W. A study of certain viruses pathogenic to the tomato. Unpublished thesis, Purdue Univ., 1932.

given not to those that the streak virus may successfully resist, but to those to which it may readily succumb. Inasmuch as mosaic A enters as a component part of the combination streak, the previous work on this mosaic agent is of some interest in connection with the inactivation of the streak virus. However, the virus of mosaic B is by far more sensitive to destructive chemicals than is virus mosaic A; therefore, when a streak-containing juice is treated, weaker solutions tend to kill virus B and stronger solutions kill both A and B. There were a few exceptions in the writer's work that, *ipso facto,* appear particularly interesting.

MATERIALS USED AND RESULTS

Acids. Four acids were tested: Sulphurous, deoxycholic, boric, and diiodo-salicylic. Sulphurous acid was prepared by the action of warm dilute sulphuric acid on sodium sulphite (Na_2SO_3). The original strength, ascertained by the method outlined by Treadwell and Hall (23) was about 6 per cent. SO_2 is recognized as a powerful antiseptic employed successfully in industry. Trautwein (22) found H_2SO_3 effective in inactivating the foot and mouth virus. It was used by the writer for the first time in connection with the inactivation of plant viruses. Deoxycholic acid was extracted from ox-gall powder by the method described by White (24) without essential changes. Besides the final product two intermediate non-conjugated acids were also tried, namely: (a) A crystalline substance obtained after the first washing in acetic acid (prior to xylene purification) and containing some fatty acids (referred to in the table as xylene-cholic acid); and (b) a crystalline substance obtained after washing in xylene but before final washing in glacial acetic acid and containing some xylene (referred to in the table as fatty-cholic acid).

As may be seen from table 1, sulphurous acid (H_2SO_3) appeared to be highly toxic to the streak complex. At the concentration of about and above 1.5 per cent both component viruses were inactivated; below that and down to about 0.66 per cent the virus of mosaic B was completely eliminated without injury to the virus of mosaic A. With still lower concentrations the effect was only partial or none at all.

Deoxycholic acid also showed a distinct toxicity, although not so phenomenal as in the case of pneumococcus, as found by Downie, Stent, and White (11). Only the 5 per cent strength of this chemical appeared to be totally fatal to mosaic B and nearly so to mosaic A. The two intermediate substances gave approximately the same results. This is the first application of deoxycholic acid to the plant-virus-inactivation work. In no case was mosaic B left active after the elimination of mosaic A. Boric acid (H_3BO_3), in the concentrations used, proved entirely ineffective. Five per

cent solution of diiodo-salicylic acid showed some toxic effect comparable with that produced by intermediary bile acids.

TABLE 1.—*Results of treating streak-infective tomato juices with acids*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
H_2SO_3					
0.18	6	All	0	0	0
0.36	6	1	4	0	1
0.65	6	0	2	0	4
0.72	6	0	4	0	2
1.3	6	0	4	0	2
1.5	6	0	0	0	All
2.65	6	0	0	0	"
3.0	6	0	0	0	"
Deoxycholic acid					
0.06	4	All	0	0	0
0.12	4	"	0	0	0
0.25	4	"	0	0	0
0.5	4	"	0	0	0
1.0	4	"	0	0	0
2.0	4	3	1	0	0
5.0	4	0	1	0	3
Xylene-cholic acid					
0.1	4	All	0	0	0
1.0	4	"	0	0	0
5.0	4	1	1	0	2
Fatty-cholic acid					
0.1	4	All	0	0	0
1.0	4	"	0	0	0
5.0	4	1	1	0	2
H_3BO_3 (Boric acid)					
2.5 and 5.0	22	All	0	0	0
Diiodo-salicylic acid ($I_2C_6H_3COOH$					
5.0	8	1	5	0	2

Sulphites. Sodium sulphite (Na_2SO_3), sodium acid sulphite ($NaHSO_3$), and potassium metabisulphite ($K_2S_2O_5$) were used. All three were employed for the first time as inactivators in the plant-virus work. None of these salts was nearly so effective as H_2SO_3. The virus of mosaic B was present to a greater or lesser degree in all the salt and plant-juice mixtures containing 2.5 and 5.0 per cent of these sulphites, whereas the virus of mosaic A showed also a partial survival, even in a 10 per cent mixture of $K_2S_2O_5$. Summarized results are given in table 2.

TABLE 2.—*Results of treating streak-infective tomato juices with certain sulphites*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Na_2SO_3					
2.5	6	All	0	0	0
5.0	6	"	0	0	0
$NaHSO_4$					
2.5	6	All	0	0	0
5.0	6	2	2	0	2
$K_2S_2O_5$					
2.5	6	5	1	0	0
2.5	6	All	0	0	0
5.0	6	2	2	0	2
10.0	6	0	2	0	4

Sulphates. A few sulphates were previously used by other workers in connection with the virus-disease work. Thus, Allard (2) used copper sulphate on tobacco-mosaic and inactivated it in a 0.2 per cent mixture. The same virus was treated by him with manganese sulphate at the strength of approximately 7.5 per cent, but the results were negative. $CuSO_4$ also was used by Doolittle (9) on cucumber mosaic, which was inactivated at 0.5 per cent. Caldwell (3) tried mercuric sulphate in acid solution in connection with tobacco mosaic and obtained inactivation, but the virus was reactivated on addition of alkali. Since tobacco mosaic (mosaic A) is a part of combination-streak complex, Allard's tests with $CuSO_4$ are to be especially noted here. In the writer's treatments of the streak-containing juice with this chemical, 2.5 and 5.0 per cent strengths were used. In all cases mosaic B was eliminated and in most cases mosaic A, also. A similar lytic action was manifested by $KHSO_4$ with which a 0.6 per cent mixture alone was not entirely effective against mosaic B, whereas mosaic A showed some activity even after the 5 per cent bath. On the other hand, K_2SO_4 at similar dilutions was entirely ineffective against any part of the complex. The most decisive inactivating action was shown by antimony sulphate and bismuth sulphate, although only one controlled experiment in each case was performed. In these experiments the streak complex was entirely inactivated by a 5 per cent solution of these salts. Field experiments also were carried on with these substances, but the plants were unprotected and naturally became contaminated. Therefore, they do not show a complete eradication of the streak complex nor of the mosaic A virus, although the effectiveness of these chemicals is clearly revealed. Sodium thiosulphate ($Na_2S_2O_3$) showed very weak lytic action.

TABLE 3.—*Results of treating streak-infective tomato juices with certain sulphates*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
$CuSO_4$					
2.5	6	0	1	0	5
5.0	17	0	3	0	14
$KHSO_4$					
0.6	6	1	5	0	0
1.25	6	0	5	0	1
2.5	6	0	6	0	0
5.0	6	0	2	0	4
5.0	6	0	0	0	All
K_2SO_4					
5.0	6	All	0	0	0
6.3	6	"	0	0	0
$Sb_2(SO_4)_3$					
Grhs.:					
5.0	13	0	0	0	All
Field:					
8.0	11	0	1	0	10
Half-saturated	12	1	8	0	3
Saturated	10	0	3	0	7
$Bi_2(SO_4)_3$					
Grhs.:					
5.0	5	0	0	0	All
Field:					
8.0	11	2	8	0	1
Half-saturated	10	6	3	0	1
Saturated	10	2	8	0	0
$Na_2S_2O_3$					
0.01– 1.0	9	All	0	0	0
5.0	4	"	0	0	0
10.0	6	"	0	0	0
15.0	6	"	0	0	0
20.0	6	4	2	0	0
25.0	6	3	2	0	1
$CoSO_4$					
5.0	14	6	1	3	4
$NiSO_4$					
5.0	6	2	0	4	0
10.0	6	3	0	3	0
10.0	6	All	0	0	0
15.0	6	2	4	0	0
20.0	6	0	2	0	4
20.0	6	1	0	2	3
25.0	6	5	0	0	1
25.0	6	2	2	0	2
$ZnSO_4$					
Grhs.:					
2.5	26	23	1	0	2
5.0	11	7	2	0	2
10.0	11	3	1	0	7
Field:					
2.5	10	6	1	0	3
5.0	15	5	6	2	2
10.0	13	0	2	1	10

Rather unexpected results were obtained with cobalt sulphate, nickel sulphate, and zinc sulphate. Their lytic effect, unlike that of other tested substances, was not much stronger with mosaic B than with mosaic A. When the solution was not strong enough to kill both components parts, either one or the other would survive. The results obtained with these sulphates to date, while of considerable interest, do not warrant a definite conclusion as to the effect of the chemicals in question, and merely suggest a possibility of splitting viruses A and B in the combination streak complex by chemical means. These results, however, cannot yet be predicted. It is possible that in the case of $NiSO_4$ the separation of the B virus was due to cobalt present as an impurity in the amount of 0.008 of 1 per cent. The experimental data are summarized in table 3. Allard (1) successfully used nickel hydroxide to inactivate tobacco mosaic, but no other nickel compounds were employed.

Bile and bile salts. Bile was used successfully by Friedberger and Yamamoto (12) for treatment of variola, by Landsteiner *et al.* (15) for poliomyelitis, and by Todd (21) for cattle plague. Apparently, the writer applied it to plant-virus-inactivation work for the first time. In these experiments it did not prove to be very toxic to the streak complex. Virus B showed some activity, even in the 20 per cent solution, and virus A in the 25 per cent solution. No healthy plant was obtained at or below 15 per cent concentration. Sodium taurocholate and sodium glycocholate were used only in 2.5 and 5.0 per cent concentrations, which showed no effect on either of the two component parts of streak (table 4). Previously, Allard (2) found that a 5-day exposure to 5 per cent sodium taurocholate solution inactivated tobacco virus, but not the exposure to 2.0 per cent solution for the same period of time. No report is known as to the use of sodium glycocholate.

Iodine. Chandler (4, 5, 6, 7) and Chandler and Miller (8) in Michigan obtained very striking results in treating blackhead in turkey with colloidal iodine, also known on the market as iodine suspensoid. This form of iodine appeared to be the most active and available form of this element. Prof. Chandler very kindly arranged with Merck and Company to supply the writer with iodine suspensoid for his experiments. It showed a decided toxicity and was lytic to mosaic B in a 1.0 per cent solution and to both component viruses in a 5.0 per cent solution. A 1.0 per cent solution of KI, however, used at the same time, showed no effect either on the virus of mosaic A or on the virus of mosaic B (table 5).

DYES, DISINFECTANTS, AND MISCELLANEOUS MATERIALS

In the work of Lewis (16, 17, 18) with various animal and fowl viruses and of Schultz and Kruger (20) with Staphylococcus bacteriophage,

TABLE 4.—*Effect of bile and bile salts on the streak virus*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Bile					
0.01– 1.0	9	All	0	0	0
2.5	4	"	0	0	0
5.0	4	3	1	0	0
10.0	6	All	0	0	0
10.0	6	3	3	0	0
15.0	6	2	4	0	0
20.0	6	2	2	0	2
25.0	6	0	5	0	1
Sodium taurocholate					
2.5	6	All	0	0	0
5.0	6	"	0	0	0
Sodium glycocholate					
2.5	6	All	0	0	0
5.0	6	"	0	0	0

methylene blue proved to be a very satisfactory inactivator even at very low concentrations. The writer made a test of a 1 per cent concentration on 3 young plants, but the results were negative. No other dyes were employed in this streak work. In the disinfectant group, mercurochrome was ineffective at and below 1.0 per cent strength, whereas lysol at 2.5 per cent showed some effect on mosaic B. Of other substances used in the concen-

TABLE 5.—*Effect of iodine preparations on the streak virus*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Iodine suspensoid					
0.1	4	All	0	0	0
0.2	4	"	0	0	0
0.5	4	"	0	0	0
1.0	4	0	4	0	0
2.0	4	0	3	0	1
5.0	4	0	0	0	All
10.0	4	0	0	0	"
10.0	6	1	0	0	5
10.0	6	0	0	0	All
10.0	6	0	0	0	"
25.0	6	0	0	0	"
50.0	6	0	0	0	"
KI					
1.0	4	All	0	0	0

trations not exceeding 5 per cent, saponin, rotenone, thiourea and cholesterol, proved to be entirely ineffective. One plant out of 12 infected with the juice containing 10 per cent of reuniol entirely escaped infection, which may, however, be accidental. A 5 per cent solution of sodium ricinolate, on the other hand, showed a definite, though moderate, effect on both mosaic A and mosaic B.

Another salt, sodium salicylate, included in this group, showed a strong toxicity to the entire streak complex in the 5 and 10 per cent mixtures and some effect on mosaic B, even at 1 per cent. Further details are given in table 6.

TABLE 6.—*Effect of certain chemicals on the streak virus*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Methylene blue					
1.0	3	All	0	0	0
Mercurochrome					
0.01–1.0	9	All	0	0	0
Lysol					
2.5	6	5	1	0	0
2.5	6	3	3	0	0
Saponin					
0.01–1.0	9	All	0	0	0
5.0	4	"	0	0	0
Rotenone					
5.0	12	All	0	0	0
Thiourea					
5.0	12	All	0	0	0
Cholesterol					
5.0	12	All	0	0	0
Reuniol (emulsion)					
10.0	12	11	0	0	1
Sodium ricinolate					
5.0	10	6	1	0	3
Sodium salicylate ($NaC_7H_5O_3$)					
1.0	4	3	1	0	0
5.0	4	0	0	0	All
10.0	4	0	0	0	"

SUMMARY

A number of chemical substances, known to produce strong lytic action on certain microorganisms, were added to unfiltered tomato juices containing the virus of combination streak. After allowing them to remain in these solutions for 2 hours, these juices were applied to young healthy tomato plants just as were untreated juices. The results were evaluated in comparison with the control plants, which in every case showed 100 per cent infection in from 4 to 5 days. The virus was considered inactivated when symptoms failed to appear within 30 days.

According to the results obtained, the chemicals used in this work fall into 3 groups: (a) Those showing little or no effect on either of the two component parts of the streak; (b) those having a decided lytic effect on both components with virus B, as a rule, being the weaker part of the complex; and (c) those that revealed no special affinity for either virus A or virus B.

The first group, in the order of ascending degree of effectiveness, was comprised of the bile and the 2 bile salts, $I_2C_6H_3COOH$, $Na_2S_2O_3$, K_2SO_4, Na_2SO_3, $K_2S_2O_5$, H_3BO_3, saponin, rotenone, thiourea, cholesterol, reuniol, and sodium ricinolate. Methylene blue, mercurochrome, and lysol may belong to this group, although these substances were tested only in weaker dilutions.

The second group, using the same order, consisted of the unconjugated bile acids, $CuSO_4$, $KHSO_4$, $NaC_7H_5O_3$, iodine suspensoid, $Sb_2(SO_4)_3$, $Bi_2(SO_4)_3$ and H_2SO_3. The latter was effective in relatively low concentrations.

The remaining tested chemicals, namely, $CoSO_4$, $NiSO_4$, and $ZnSO_4$, especially the first two, showed a lytic effect on both components of the streak complex but, unlike other substances tested, this effect was little stronger on the virus of mosaic B than on that of mosaic A, since, when the solution was not strong enough to kill both viruses, sometimes one and sometimes the other would survive. The data, however, are not sufficient to predict the results of such treatments.

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THE INHERITANCE OF RESISTANCE TO COMMON BEAN MOSAIC IN FIELD AND GARDEN BEANS[1]

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An investigation of the mode of inheritance of mosaic resistance was undertaken coincidental with the development of 2 mosaic-resistant varieties of Refugee type beans. These resistant varieties of Refugee, Wisconsin Refugee, and Idaho Refugee, were developed by Pierce and Walker (7) from crosses of the regular Refugee Green variety with the resistant variety Corbett Refugee.

It was noted that in these crosses the F_1 hybrids were resistant to mosaic. McRostie (2) had previously found that in various crosses in which the Robust variety was used as the resistant parent, susceptibility was at least partially dominant. Also, Parker and Brink (5) in studying Robust-Refugee Green crosses, found that in F_2 progenies 56 per cent of the plants were resistant in crosses in which Robust was used as the female parent and that only 1.3 per cent of the plants were resistant after the reciprocal cross. It was apparent, therefore, that in all probability the resistances of Corbett Refugee and Robust were inherited differently and that the whole problem needed further study. In the present investigation the inheritance of mosaic resistance of 3 resistant varieties, Corbett Refugee, Great Northern UI No. 1, and Robust was studied in crosses with the susceptible Refugee Green variety. This paper presents the results obtained from an analysis of the F_1 and F_2 generations of these crosses.

EXPERIMENTAL MATERIALS AND METHODS

The following crosses were made in the greenhouse at Madison, Wisconsin, beginning in the fall of 1930:

(R)[3] Corbett Refugee[4] × (S) Refugee Green (and reciprocal).

(R) Great Northern UI No. 1 × (S) Refugee Green (and reciprocal).

(R) Robust × (S) Refugee Green.

(R) Corbett Refugee × (R) Great Northern UI No. 1 (and reciprocal).

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[3] (R) designates variety resistant to common bean mosaic.
(S) designates susceptible variety.

[4] The female parent is written first and the male parent second in all crosses.

(R) Corbett Refugee × (R) Robust.

(R) Great Northern UI No. 1 × (R) Robust.

These crosses were made on greenhouse-grown, mosaic-free plants. The 4 varieties used were among those tested (6) for resistance and susceptibility to common bean mosaic. Greenhouse inoculation trials had shown that these varieties were either homozygous for resistance or for susceptibility. Especial mention is due Mr. R. D. Corbett who developed the Corbett Refugee variety from a single mosaic resistant plant found in a heavily infected stand of Refugee Green beans. He very kindly furnished seed for this investigation. In figure 1 is shown the mosaic resistant Corbett Refugee variety and

FIG. 1. Field plantings of two bean varieties used in various hybrids. Mosaic-resistant Corbett Refugee variety at left. Mosaic-infected Refugee Green variety at right.

the mosaic-infected Refugee Green variety grown in the field trials in 1934.

The bean mosaic virus used was the common bean mosaic virus (*Bean virus 1*) originally described by Reddick and Stewart (8, 9, 10) and shown by them to be seed-borne and to be nontransmissible to the Robust variety. The susceptibility of the F_1 and F_2 progenies to this virus was determined in the field and greenhouse at the University of Wisconsin, during 1931 and 1932, and at the University of Idaho during 1933 and 1934. All progenies were artificially inoculated by rubbing the young primary leaves with infected leaf material. The field trials were, in addition, subject to infection from natural sources.

It was previously shown (6) that the yellow bean-mosaic virus (*Bean virus 2*), which may overwinter on sweet clover, will affect many varieties of the common bean, including varieties resistant to the common form of bean mosaic. Since the field symptoms of both types of mosaic are readily confused, it was imperative that natural infections with the yellow form be

ruled out. This was done by making transfer inoculations from plants suspected of being affected with *Bean virus 2* to differential varieties in the greenhouse. In this manner natural infections with yellow bean mosaic were effectively determined and not confused with infections of common bean mosaic.

Also, it was found that certain chlorophyll-deficient segregants of the type described by Burkholder and Muller (1) and by Parker (3, 4) might readily be confused with mosaic symptoms. It was felt necessary, therefore, to test plants of this type by making transfer inoculations to known susceptible plants in order to determine whether or not they were actually infected with mosaic. This added considerably to the task of classifying progenies; but the actual testing showed its necessity, for it was found that while some chlorophyll-deficient plants were resistant, others were actually infected with mosaic but with the symptoms so masked that the infection was impossible of detection without an actual test.

The F_2 tests were made only with seed obtained from F_1 plants that had been tested for mosaic reaction. Thus in crosses found to be susceptible in F_1, there were a certain number of plants showing primary infection in F_2. Those F_2 plants arising from mosaic-infected seed were of necessity classified as susceptible plants.

Seed of all progenies was scarified before planting to insure uniform and complete germination. Inoculations were made as soon as the young primary leaves were fully expanded. As soon as symptoms appeared in the F_2 progenies, the infected plants were counted and removed; the remaining healthy plants were then reinoculated so as to insure the infection of all susceptible individuals. Plants were removed as soon as they showed unmistakable symptoms in order to insure accuracy in classification, since the mosaic symptoms tend to become masked as the plants grow older.

EXPERIMENTAL RESULTS

F_1 Progeny Tests

The F_1 generation of the crosses listed in table 1 were all grown in the field and were artificially inoculated with the virus of common bean mosaic besides being subject to infection from natural sources. Crosses in which Corbett Refugee was involved were resistant to mosaic in the F_1 generation. Thus hybrids of (R) Corbett Refugee with (S) Refugee Green, with (R) Great Northern UI No. 1 and with (R) Robust were all resistant. Also the cross, Great Northern UI No. 1 × Robust was resistant. However, hybrids of (R) Robust and (R) Great Northern UI No. 1 with (S) Refugee Green were found to be susceptible. There was no apparent difference in susceptibility to mosaic of reciprocal crosses involving the same parents in the first generation.

TABLE 1.—*Occurrence of common bean mosaic in the F_1 and F_2 progenies of certain reciprocal bean hybrids when inoculated with the virus of common bean mosaic*

Parentage of crosses[a]	Total F_1 plants tested	F_1 mosaic reaction	Segregation in F_2 Resistant: Susceptible	Resistant F_2 plants
				Per cent
(S)[b] Refugee Green No. 109 × (R) Corbett Refugee	31	resistant	1688: 361	82.3
(S) Refugee Green No. 108 × (R) " "	6	"	278: 69	80.1
(S) Refugee Green No. 114 × (R) " "	11	"	275: 66	80.6
(S) Refugee Green No. 112 × (R) " "	7	"	198: 32	86.1
TOTALS	55	resistant	2439: 528	82.2
(R) Corbett Refugee × (S) Refugee Green No. 109	40	resistant	2111: 262	88.9
(R) " " × (S) Refugee Green No. 108	3	"	115: 15	88.4
(R) " " × (S) Refugee Green No. 114	5	"	89: 11	89.0
(R) " " × (S) Refugee Green No. 112	3	"	91: 15	85.8
TOTALS	51	resistant	2406: 303	88.8
(S) Refugee Green No. 109 × (R) Grt. Northern UI No. 1	19	susceptible	57: 316	15.2
(R) Grt. Northern UI No. 1 × (S) Refugee Green No. 109	9	"	82: 375	17.9
(R) Robust × (S) Refugee Green No. 109	3	susceptible	82: 598	12.0
(R) Great Northern UI No. 1 × (R) Robust	4	resistant	59: 0	100.0
(R) Corbett Refugee × (R) Grt. Northern UI No. 1	7	"	47: 6	88.6
(R) Grt. Northern UI No. 1 × (R) Corbett Refugee	16	"	141: 16	89.8
(R) Corbett Refugee × (R) Robust	8	"	95: 25	79.2
(R) Idaho Refugee × (S) Refugee Green No. 109	1	"	81: 13	85.9
(S) Refugee Green No. 109 × (R) Idaho Refugee	2	"	91: 13	87.5
(R) Wisconsin Refugee × (S) Refugee Green No. 109	2	"	159: 28	85.0

[a] The female parent is written first and the male parent second in all crosses.
[b] S designates variety susceptible to common bean mosaic.
R designates resistant variety.

The fact that Corbett Refugee gave a different reaction in F_1 in crosses with Refugee Green than did Robust or Great Northern UI No. 1 with Refugee Green, is alone sufficient evidence to indicate that Corbett Refugee possesses a different type or degree of resistance. Further evidence, however, is to be found by an examination of the F_2 results.

F_2 Progeny Tests

Corbett Refugee × Refugee Green. As shown in table 1, Corbett Refugee was crossed with 4 different susceptible strains of Refugee Green. Offspring of crosses of Corbett Refugee and Refugee Green strain No. 109 contained 6.6 per cent more resistant plants when the susceptible variety (Refugee Green) was used as the pollen (♂) parent than when used as the pistillate (♀) parent. Refugee Green strains No. 108 and No. 114 gave similar results. There was no significant difference in reciprocal crosses of the Refugee Green strain No. 112 with Corbett Refugee.

A summary of the data of all 4 strains of the cross Refugee Green (♀) × Corbett Refugee (♂) shows that 2439 individuals were classified as resistant and 528 as susceptible. The expectation on the basis of a 13:3 ratio would be 2410.7 resistant to 556.3 susceptible. However, a summary of the data on the reciprocals of these crosses shows 2406 classified as resistant and 303 as susceptible, which is too far from a 13:3 expectancy to be significant. It is possible that certain cytoplasmic elements may in some manner have suppressed expression of the susceptibility of 1/16 of the population in the latter case.

In view of the fact that there is a difference of 6.6 per cent resistance between reciprocal crosses, suggesting that the inheritance of resistance may not be entirely Mendelian, no factorial explanation for the results secured is attempted.

Great Northern UI No. 1 × Refugee Green. An analysis of the F_2 generations of reciprocal crosses of Great Northern UI No. 1 and Refugee Green shows that from 15 to 18 per cent of the plants were resistant (Table 1). Figure 2 shows the relative number of mosaic-resistant plants obtained in the F_2 generation of this cross compared to a cross of Great Northern UI No. 1 with Corbett Refugee. The 15 to 18 per cent resistant plants obtained in the Great Northern UI No. 1–Refugee Green cross is much lower than the 82 to 89 per cent found in the Corbett Refugee crosses with Refugee Green. This result, however, was to be expected, since the Great Northern UI No. 1 hybrids were found susceptible in F_1 and the Corbett Refugee hybrids resistant. The reciprocals of the Great Northern UI No. 1–Refugee Green cross show a divergence of only 2.7 per cent resistant plants.

Robust × Refugee Green. A cross of Robust × Refugee Green No. 109 in which Robust was used as the female parent has been analyzed. As shown

FIG. 2. Relative number of mosaic-resistant plants obtained in F_2 generation of certain crosses. The susceptible plants had been removed. Great Northern UI No. 1 × Corbett Refugee at left. Great Northern UI No. 1 × Refugee Green at right. Note that relatively few plants were resistant.

in table 1, an F_2 population of 680 plants was found to contain only 82 resistant plants or 12 per cent. The reciprocal cross was not studied. However, even if no resistant plants were to be obtained in the reciprocal, there could not be so wide a difference between reciprocal crosses as was reported by Parker and Brink (5). In a similar cross they found 56 per cent resistant plants in F_2 when Robust was used as the female parent and only 1.3 per cent after the reciprocal cross. Obviously, the data are somewhat conflicting; but it is possible that the strain of Robust or strain of Refugee Green used in the 2 investigations differed genetically. However, as shown in the results obtained with the Corbett Refugee–Refugee Green crosses, there was little observable difference among the 4 different strains of Refugee Green used in this investigation.

Crosses Between Resistant Varieties. It is interesting to note that segregation of susceptible individuals was obtained in the F_2 generation of crosses between certain resistant varieties (Table 1). Corbett Refugee × Great Northern UI No. 1, both resistant varieties, segregated between 10 and 12 per cent susceptible individuals in F_2 with no appreciable difference in reciprocal crosses (Figure 2, Left). The percentage of susceptible individuals segregating in this cross is practically the same as in the cross of Corbett Refugee (♀) × Refugee Green (♂). It is probable that the resistant

Great Northern UI No. 1 variety carries factors for susceptibility as well as for resistance.

Also, a Corbett Refugee × Robust cross segregated susceptible individuals in F_2. In an F_2 population of 120 individuals 25 were found susceptible (Table 1). This result might well have been predicted from the results obtained in the crosses of Robust × Refugee Green, Great Northern UI No. 1 × Refugee Green, and Corbett Refugee × Great Northern UI No. 1. That is, since Robust and Great Northern UI No. 1 behaved similarly in crosses with Refugee Green, it would be expected that Robust–Corbett Refugee crosses would segregate similarly to the Great Northern UI No. 1–Corbett Refugee cross. The probability that the resistances of Robust and Great Northern UI No. 1 are similar is further indicated by the results of a cross of these 2 varieties. A small progeny of 59 individuals of a Robust–Great Northern UI No. 1 cross did not segregate any susceptible individuals (Table 1).

However, it should be pointed out that Great Northern UI No. 1 appears to have a slightly higher degree of resistance, as evidenced by other crosses, than does Robust. Thus, the F_2 of the Great Northern UI No. 1 × Refugee Green cross segregated 17.9 per cent resistant individuals, while Robust × Refugee Green segregated only 12 per cent resistant individuals. And furthermore, the crosses of Robust and Great Northern UI No. 1 with Corbett Refugee, in which the Robust crosses segregated 20.8 per cent susceptible plants, and the Great Northern UI No. 1 crosses segregated 11.4 per cent, indicate that Robust is slightly less resistant genetically.

These differences are not great, especially when compared to the high degree of resistance of Corbett Refugee, and since the cross of Great Northern UI No. 1 × Robust failed to segregate, it would appear that any difference in the resistances of these two varieties might well be attributed to modifying factors.

Wisconsin Refugee and Idaho Refugee × Refugee Green. Pierce and Walker (7) demonstrated the practicability of using Corbett Refugee as a parent in the development of mosaic-resistant Refugee beans. The 2 varieties, Wisconsin Refugee and Idaho Refugee, which they developed from the cross of Refugee Green (♀) with Corbett Refugee (♂), were used in back crosses to Refugee Green. The F_2 segregation shown in table 1 indicates that the 2 new varieties possess approximately the same degree of resistance as the original resistant parental variety, Corbett Refugee.

This is evidence that, even though a slightly less number of resistant plants were obtained in the original Refugee Green (♀) × Corbett Refugee (♂) cross than in the reciprocal, it was still possible to obtain F_2 plants with the same degree of resistance as the resistant pollen parent.

DISCUSSION

The significance of the present investigations is largely in showing that certain mosaic resistant varieties of the common bean possess a different type or degree of resistance, and that it is possible to predict with a reasonable degree of accuracy the results that may be expected from crosses of these various resistant varieties with susceptible types.

Other than the slight difference found in the reaction of certain reciprocal crosses, the results on the whole were quite consistent. Thus, Great Northern UI No. 1, showed a tendency to give slightly more resistant individuals in F_2 than did Robust, both in crosses with Refugee Green and with Corbett Refugee. Furthermore, Corbett Refugee has consistently demonstrated a dominant type of resistance, as shown by resistant F_1 hybrids followed by a high percentage of resistant plants in F_2 populations.

It is believed that the results are sufficiently consistent to allow for a fairly accurate prediction of what may be expected of these resistant varieties in crosses with susceptibile types. This is, after all, the primary value of a genetical study insofar as the practical plant breeder is concerned, regardless of whether or not a strict factorial explanation is advanced. No explanation on a factorial basis is attempted because of the difference obtained in certain reciprocal crosses, which suggests that the inheritance of resistance may in part be non-Mendelian. However, since the differences in F_2 were found to be relatively slight, and since F_1 was not matroclinous, we are not inclined to agree wholly with the suggestion of Parker and Brink (5) that the basis of resistance is something probably carried in the cytoplasm. It would appear from our results that more probably specific genes are responsible for resistance, and that they may be influenced somewhat by the cytoplasm with which or within which they are acting.

SUMMARY

The studies reported in this paper were concerned with the inheritance of resistance to common bean mosaic in crosses involving three resistant varieties, Corbett Refugee, Robust, and Great Northern UI No. 1, and one susceptible variety, Refugee Green.

All hybrids involving Corbett Refugee were found to be resistant to common bean mosaic in the F_1 generation. The F_1 generation of crosses of Great Northern UI No. 1 and Robust with Refugee Green were susceptible.

In the F_2 generation the Corbett Refugee–Refugee Green hybrids segregated 89 per cent resistant plants when Corbett Refugee was used as the male (pollen) parent and 82 per cent after the reciprocal cross. The F_2 progenies of Great Northern UI No. 1 × Refugee Green segregated 15 to 18 per cent

resistant plants, and Robust × Refugee Green segregated 12 per cent resistant plants.

Crosses between certain resistant varieties were found to segregate susceptible plants in F_2. Corbett Refugee × Great Northern UI No. 1 segregated 11 per cent susceptible plants; and Corbett Refugee × Robust segregated 20.8 per cent susceptible plants. Great Northern UI No. 1 × Robust did not segregate in F_2, indicating that the resistances of these two varieties were similar. Two homozygous resistant varieties, Idaho Refugee and Wisconsin Refugee, selected from the Refugee Green (♀) × Corbett Refugee (♂) cross, were backcrossed to Refugee Green. An F_2 analysis showed that these 2 resistant varieties possessed the same degree of resistance as the original resistant parent variety, Corbett Refugee.

It was concluded that the data obtained from the various crosses were consistent enough to allow for fairly accurate predictions of what might be expected of the resistant varieties studied when crossed with other varieties.

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PHYTOPATHOLOGICAL NOTES

Areolate Spot of Citrus Caused by Leptosphaeria bondari.—The known range of areolate spot, a striking leaf and twig disease of citrus (*Citrus* spp.), so named and discussed by Bondar[1] as occurring in Bahia, Brazil, has recently been extended to Dutch Guiana and Venezuela. This information is based upon specimens at hand (Fig. 1, A and B) from Paramaribo, Surinam, Dutch Guiana (C. Stahel, Oct. 7, 1933), and from San Cristobal, Táchira, Venezuela (I. J. Otera, Oct. 28, 1933), sent by C. Chardon and Stahel, respectively to H. S. Fawcett, from whom they were received as then unidentified. In southern Brazil records of the disease in the Federal District and São Paulo were recently reported,[2] and during the past year specimens of diseased leaves have been received from Morretes, Paraná (H. Grillo, Feb. 15, 1934).

Areolate spot, which is particularly destructive in the nursery, is apparently a disease of moist warm climates. Both Bondar and Stahel[3] refer to it as prevalent in the nursery during the rainy season. Stahel[3] wrote that during this period nursery plants become entirely defoliated, but that they recover during the dry season. The disease has not been observed in the plantations in the highlands of São Paulo, where most of the citrus groves are located.

On the lesions of areolate spot, the symptoms of which are described by Bondar, he found 3 forms of what he interpreted to be the same fungus, 2 pycnidial, one with conidia 2–3 $\mu \times 4\ \mu$, and the other with 4-septate, hyaline conidia 20 μ in length, and a Leptosphaeria. He does not state the color of the smaller conidia, but those observed in the specimens examined, including those from Bondar, are hyaline, becoming distinctly colored (Fig. 1, C and E). This stage, therefore, may be considered a Coniothyrium, which genus generally is recognized as the conidial stage of Leptosphaeria. At São Paulo monosporous cultures were made from these 2 stages as present on a specimen contributed by Bondar (Nov. 1932, Bondar 337) on which only these 2 stages were found. The 2 isolations were of similar appearance; Hendersonia, or what is possibly the septate conidial form referred to by Bondar, was obtained from the ascospore culture, while the conidial culture yielded only Coniothyrium. Inoculations were made with both cultures, but no definite results were obtained.

[1] Bondar, G. Insectos daminhos e molestias da laranjeira no Brasil. Bol. Lab. Path. Veg. Bahia, 7, 79 pp. 1929.

[2] Bitancourt, A., J. P. Fonseca, and M. Autuori. Manual de Citricultura, II Parte. Doencas, pragas e tratamentos. 212 p. Edição da "Chacaras e quintaes," São Paulo. 1933.

[3] Letter to H. S. Fawcett, dated Oct. 7, 1933.

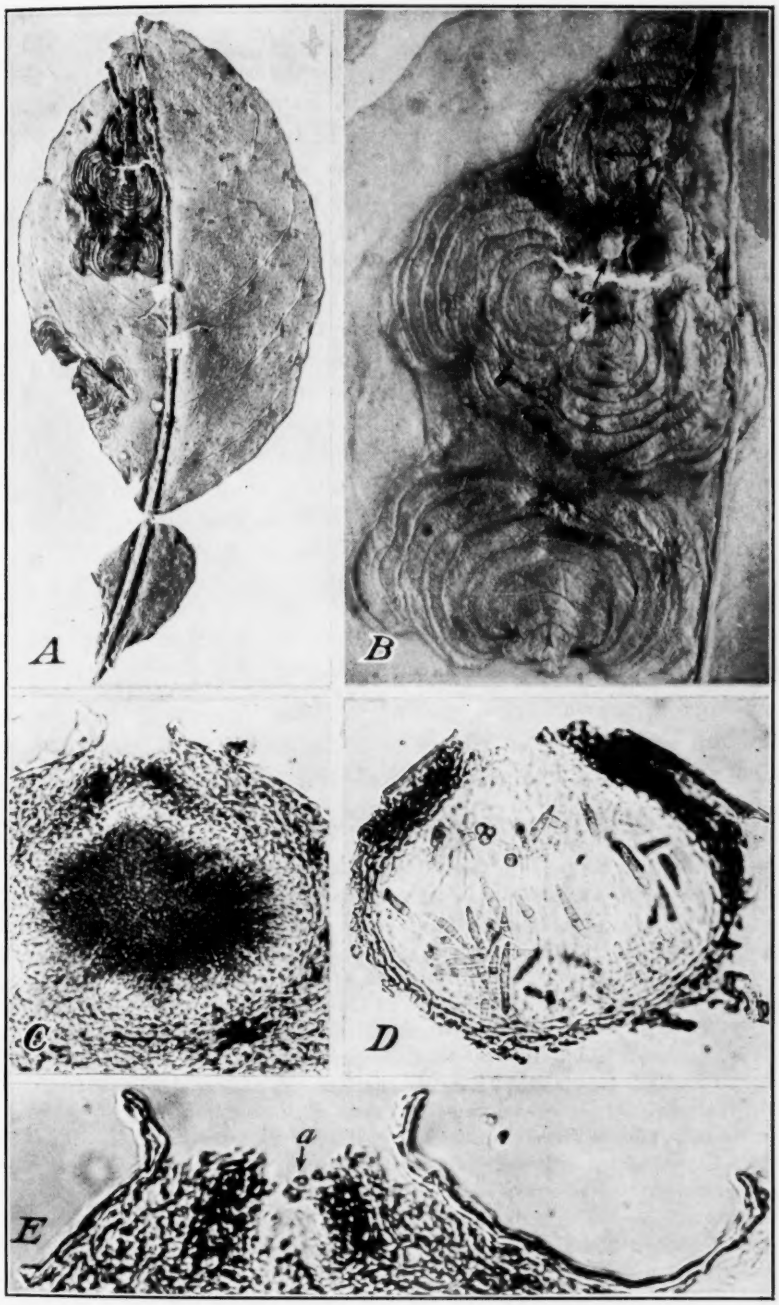

FIG. 1. A. Areolate spot of citrus on what is apparently sour orange, from Surinam, Dutch Guiana, collected by G. Stahel (x 1). B. Upper surface of large lesion shown in A (x 4); *a*, initial lesions reported by Bondar for the disease in Bahia; *b*, Coniothyrium pycnidia. C–E. Coniothyrium pycnidia from A, showing (C) colored conidial mass and (E, *a*) individual conidia in ostiole and (D) a perithecium of the Leptosphaeria from the specimen from Bondar referred to in the text (C × 325; D, × 380; E, × 500).

In attributing areolate spot to the Leptosphaeria, Bondar states that the disease is evidently not new. He cites an instance in which it was said to have been destructive in orange nurseries in Bahia 10 years earlier. Moreover, he considered that the same disease was reported in São Paulo as *Phyllosticta hesperidearum* Catt., as well as in Ceylon, as *P. disciformis* Penz. No citations are given, but careful search of the literature to which he may have had reference fails to disclose a distinctive disease corresponding to the description of areolate spot.

Bondar identified the Leptosphaeria as *L. citricola* Penz., which is described as occurring in Italy, but with no mention of the striking areolate spot of South American disease. In the writers' opinion areolate spot, clearly diagnosed by Bondar, is probably caused by a hitherto undescribed species of Leptosphaeria indigenous to South America, and as yet unknown elsewhere. Primarily, on the basis of Bondar's description and illustrations of the fungus, it is, therefore, here described under the name *L. bondari*.[4]—A. A. BITANCOURT, Instituto Biologico de São Paulo, Brazil, and ANNA E. JENKINS, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Transfer of Diaporthe umbrina to the Genus Cryptosporella.—The fungus causing brown canker, one of the most important diseases of the rose in the eastern United States, was described as *Diaporthe umbrina* Jenkins[1] in 1918 and has since been so designated. Prior to the finding

[4] Technical description:

Leptosphaeria bondari, n. sp.

Asci 60–70 μ, in length, 8-spored, spores 3–5-septate, 21–24 μ long, becoming constricted at the septa, brown when mature; Coniothyrium pycnidia, spherical, somewhat flattened, 60–160 μ in diam., ostiole circular, surrounded by a darker color than the rest of the pycnidium, conidia 4 × 2–3 μ; similar pycnidia on the lesions, apparently also of this fungus, bear 4-septate conidia 20 μ in length; these were hyaline, although similar pycnidia from an isolation made from an ascospore of the Leptosphaeria yielded colored conidia.

Ascis 60–70 μ longis; sporis 3–5-cellulatis, 21–24 μ longis, demum ad septum constrictis; pycnidiis Coniothyrii 60–161 μ in diam.; conidiis 4 × 2–3 μ; conidiis forsan alius status conidici 4-septatis, 20 μ longis, hyalinis vel videtur demum coloratis.

Distribution: On leaves and twigs of *Citrus* sp., *C. aurantium* L., *C. grandis*, Osb., *C. sinensis* Osb. (Bahia Navel), also said by Bondar to occur on *C. limonia* Osb. and *C. medica* L., associated with and evidently producing the disease known as "areolate spot"; lesions reaching nearly 4 cm. in diam., initial spot about 1 mm. in diam., white and translucent, increasing progressively on one side, the dead tissue dividing into concentric ridges or zones, mature spots often pale at the center, and brown toward the outside, region surrounding zonation often conspicuously yellowed.

Type locality and type specimen: Brazil, Bahia, Bahia, on *Citrus sinensis* Osb., Nov. 1932, G. Bondar 337. In Herb. Phytopath., Inst. Biol. de São Paulo, Brazil, and in Mycol. Coll., Bur. Plant Indus., U. S. Dept. Agr., Washington, D. C.

Associated fungi: *Colletotrichum gloeosporioides* Penz., *Vermicularia* sp., and others.

[1] Jenkins, A. E. Brown canker of roses, caused by *Diaporthe umbrina*. Jour. Agr. Res. 15: 593–599. 1918.

of pseudoseptate spores, the very similar genus Cryptosporella was selected for its generic classification.[2] This genus had been chosen by Shear[3] for a similar fungus, also of somewhat indeterminate position and closely related to Diaporthe. Described as *C. viticola* Shear, this fungus possessed unicellular ascospores and a conidial stage typical of the genus Phomopsis, with both alpha and beta conidia. *Diaporthe umbrina*, on the other hand, produces conidia corresponding to the alpha type only in Phomopsis. In general these occur in irregular chambers within an ectostroma and may be more or less exposed at maturity. This "*Myxosporium*" type of fruit body is similar except for the shorter conidia, to the "*Fusicoccum*" type reported by von Höhnel[4] for several species of Cryptosporella.

The pseudoseptate spores in *Diaporthe umbrina* were observed in the spring of 1918, the second season the fungus was studied, in the National Rose Test Garden, Arlington Experiment Farm, Rosslyn, Virginia, where it was extremely abundant. The canker on which these spores were borne was apparently not of recent development and may have resulted from activity of the fungus during the preceding winter months. It was anticipated that ascospores showing more or less definite septation would be found frequently without making and special search for them. The fungus was, therefore, classified in the genus Diaporthe with explanation as to the absence of definite septation in the spores. But in the course of subsequent studies of the organism,[5,6,7] during which many specimens showing the perfect stage have been examined, unicellular spores have been the rule without exception.

It is likely that the genus Cryptosporella as now delimited includes several groupings, one of which would be the short, fusoid-spore type of Cryptospora and another the nonseptate-spore type of the rather heterogeneous genus Cryptodiaporthe. Inasmuch as certain species of Cryptodiaporthe are tardily septate, the general structure, occasional septation or near-septation, and the conidial stage of *Diaporthe umbrina* would suggest that this species would fall in this latter grouping. Under the circumstances, as has already been indicated,[8] *Diaporthe umbrina* is be-

[2] Unpublished data.

[3] Shear, C. L. The ascogenous form of the fungus causing dead-arm of the grape. Phytopath. **1**: 116–119. 1911.

[4] Höhnel, F. von. Mycologische Fragmente. Ann. Mycol. **16**: 106–109. 1918.

[5] Jenkins, A. E. Brown canker of the rose. Amer. Rose Ann. **1927**: 161–182. 1927.

[6] ———. Development of brown canker of roses. Jour. Agr. Res. **42**: 293–299. 1931.

[7] ———, and R. P. White. Identification of *Diaporthe umbrina* on rose from England. Mycologia **24**: 485–488. 1932.

[8] Wehmeyer, L. E. The genus Diaporthe Nitschke and its segregates. 349 pp. Univ. of Michigan Press, Ann Arbor. 1933.

lieved to be of the genus Cryptosporella and is here transferred to that genus as **C. umbrina,** (Jenkins) n. comb.

A comparison of the Diaporthe on rose petals shown in figure 1, A,

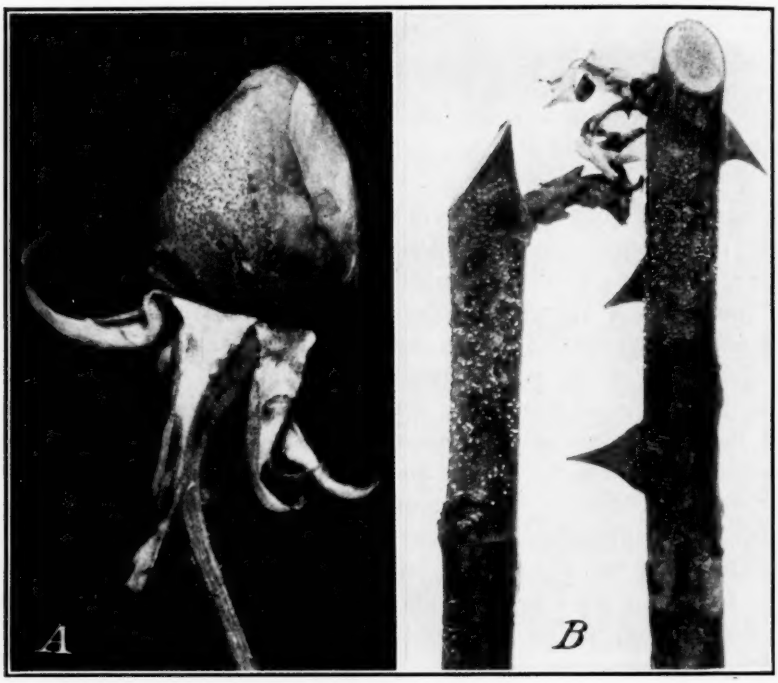

FIG. 1. Phomopsis on rose. A. On petals from Baton Rouge, La., May, 1924, C. W. Edgerton × approximately 1. B. On cuttings of *Rosa* sp. (var. La Guirlande) from a greenhouse, Arlington Experiment Farm, Rosslyn, Va., May, 1923, A. E. Jenkins. ×1.

with *Cryptosporella umbrina,* serves to illustrate the very close relationship between these 2 genera or species. The specimen represented was contributed by C. W. Edgerton in May, 1924, following the senior writer's request for material from Louisiana that might prove to be *C. umbrina.* Although the pycnidia on the petals were somewhat more prominent than is usual for corresponding fruit bodies of the Cryptosporella on this substrate, the alpha-type conidia were practically indistinguishable from conidia of the Cryptosporella. Only by a critical cultural study of the Diaporthe was it recognized as distinct from the Cryptosporella. In culture the Diaporthe grew somewhat more luxuriantly than does the Cryptosporella; moreover, both alpha and beta conidia were produced, and on corn-meal media in Erlenmeyer flasks the perfect stage developed. In

culture the latter stage in the case of the Cryptosporella has been observed only on rose-stem substrates. The asci and spores of the Diaporthe, however, were essentially indistinguishable from those of the Cryptosporella, *i.e.*, until the spores of the former became septate, which soon occurred.

The Diaporthe just discussed was previously reported without specific identification,[9] but it is here recognized as *D. eres* Nit., as broadly delimited by Wehmeyer in the monograph already cited. Several other specimens of Phomopsis from rose cankers from several different sources have been studied and cultured, more or less directly in connection with the investigation of *Cryptosporella umbrina*, but none of them has produced a perfect stage. One of these specimens is shown in figure 1, B. On the basis of their alpha and beta conidia, at least 2 of the strains are morphologically distinct from that of *D. eres* from Louisiana; nevertheless, it is probable that they all represent this same species complex.—ANNA E. JENKINS AND LEWIS E. WEHMEYER, Division of Mycology and Disease Survey, Bureau of Plant Industry, Washington, D. C., and Department of Botany, University of Michigan, Ann Arbor, Mich.

[9] U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. Sup. 42, p. 360. 1925.

REPORT OF THE NINETEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The nineteenth annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of California at Los Angeles, June 26–27, 1935, in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies.

Four half-day sessions were held. At a short business meeting that preceded the presentation of papers, C. E. Owens of Oregon State College, Corvallis, Oregon, was elected President for the ensuing year. All other officers will continue to serve until 1936, when a general election will be held at Seattle, Washington, the next meeting place of the Society.

The attendance was not so large as anticipated, the average being only 30 members at each of the sessions. Forty people signed the register, the great majority of whom were members of the Society. Twenty-four papers were presented, the titles and abstracts of which accompany the present report.

Preceding the opening session of our society, the pathologists met with The Botanical Society of America, Pacific Section; The Society for Experimental Biology and Medicine, Pacific Coast and Southern California Branches, and The Western Society of Naturalists, at a joint symposium on "Virus Diseases of Plants and Animals," which was both eminently successful and instructive. C. W. Bennett of the U. S. D. A., Riverside, was presented by the pathologists as their speaker, and his paper entitled, "The Relation of Viruses to Plant Tissues," was a distinct credit to him and the Society that he so ably represented. Dr. Bennett did not make the mistake of attempting to cover the entire subject of virus diseases as they affect plants and thereby run the danger of presenting a paper too sketchy to be of value, but rather confined himself sharply to a single phase of it—a phase in which so much greatly diversified and controversial opinion exists. His digest of the available evidence bearing on his subject was not only complete but arranged to show the fields in which experimental data are still lacking or insufficient. He clarified certain existing misconceptions of the relationship between viruses and certain plant tissues, and drew deductions from existing evidence that were at once properly conservative and logical. Lastly, he offered many meaty suggestions to future workers in this very important field of plant diseases.

Following the symposium an hour was devoted to a demonstration of techniques applicable to virus-disease investigations by various members of

the societies that had participated in the symposium. The pathologists were well represented at this session by Messrs. Carsner, Thornberry, and Takahashi, who gave practical and interesting demonstrations of various methods of plant inoculation, the preparation of virus filters, the use of stream double refractions, in the study of virus particles as well as other techniques.

B. A. RUDOLPH, *Secretary-Treasurer*

Experiments on the Multiplication of Curly-Top Virus in the Beet Leaf Hopper.—J. H. FREITAG AND H. H. P. SEVERIN.

The Effect of Carbon Dioxide on the pH Gradient of the Sugar Beet and on the Feeding of the Curly-Top Vector.—J. M. FIFE AND V. L. FRAMPTON.

The Effect of Clean Fallow on Field Populations of the Root-Knot Nematode Heterodera marioni.—J. M. OLIVEIRA.

California Celery Mosaic Diseases.—HENRY H. P. SEVERIN AND JULIUS H. FREITAG.

Two celery mosaic diseases with different symptoms and host ranges occur in California. Common names proposed for these diseases are western celery mosaic and celery calico.

The symptoms of western celery mosaic are similar to those of southern celery mosaic (Celery virus 1) but the viruses differ in host ranges. Celery calico is readily distinguished in the field by the green and amber mottling on the outer leaves, rarely on the younger leaves.

Ten species of aphids are vectors of western celery mosaic and celery calico; five of these species are known to breed on celery under natural conditions. The melon aphid, *Aphis gossypii*, transmits the 2 celery-mosaic viruses by contamination of mouth parts; each virus was transmitted by single, previously noninfective, melon aphids after feeding 5 minutes on diseased plants and then 5 minutes on healthy plants. An undetermined species of aphid belonging to the genus Aphis, tested singly, transmitted the virus of western celery mosaic by internal mechanical transmission in from 4 to 7 hours but not daily to healthy plants when transferred. Large populations of different species of aphids reared on western-celery-mosaic plants and transferred daily to healthy celery plants transmitted the virus during the first day but not on the following days.

A New Blight of Pea in California.—WILLIAM C. SNYDER.

Following a wet spring and other conditions favoring infection and spread of certain foliage diseases, severe blighting of the leaves, stems, and pods of pea, *Pisum sativum*, was observed in several market-pea areas of California early in the 1935 season. Although the principal causes of blight were found by isolation, inoculation, and taxonomic studies to be *Mycosphaerella pinodes* (Berk. and Blox.) Stone, *Ascochyta pinodella* Jones, and *A. pisi* Lib., an additional and apparently undescribed fungus differing both macroscopically and microscopically from the foregoing, was isolated and proved in inoculation trials with monosporal cultures to be actively parasitic. The new disease occurs on the leaves, stems, and pods of the common market varieties so far observed, with symptoms somewhat resembling those caused by *M. pinodes*. In pure culture the fungus produces large, black pycnidia abundantly, but on oat agar has not been found to yield the conidial exudate generally produced by the known blight organisms. Pycnidial exudate from

stems is composed of predominately nonseptate, nonconstricted, hyaline conidia that approximate or exceed in size pycnospores of *A. pisi* or *M. pinodes*. Septate conidia have been found infrequently, even at the time of their germination.

An Experiment to Determine the Susceptibility of Flax to Verticilliosis.—B. A. RUDOLPH.

The production of flaxseed and linseed oil is an industry comparatively new to California and is increasing rapidly at several points in the State. Recently the question arose as to the suitability of flax as a rotation crop to follow cotton on those soils where verticilliosis has become a problem. Apparently there is no published report of the disease in flax. It has never been recognized in flax fields in California. At the University of California Deciduous Fruit Station at San Jose, pots of steam-sterilized soil were inoculated with varying quantities of the pseudo-sclerotia of *Verticillium albo-atrum* R. & B., originally isolated from cotton and produced by the fungus on Czapeek's agar. Disinfected flaxseed was planted in these pots. Suitable checks were provided. The plants grown in pots of inoculated soil were sickly and remained stunted. The bolls matured earlier and were smaller than those of healthy plants. The leaves of the diseased plants also were smaller and turned yellow and died early. The symptoms of the sick plants were generally those ordinarily presented by other plants affected with verticilliosis. No internal discoloration of the main stems was noted, however, which may or may not have been occasioned by the conditions under which the experiment was conducted. The fungus was isolated from the main stem of a number of diseased plants an inch or more above ground. This preliminary experiment indicates that flax (Punjab) might, if planted in soils in which *Verticillium albo-atrum* has become established through the medium of cotton, contract verticilliosis.

To Visualize a Distinction between Viruses and Organisms.—J. LEE HEWITT.

Particles in a fluid medium are in motion. We may, by way of hypothesis, say that rhythmic motion of the ultimate particles of living matter is the determinant of life. If a particle of material should be of such structure, with such a distribution of masses and such a security of chemical linkage that it could be set into rhythmic motion and maintain its identity, the rhythmic motion would tend to persist at an optimum of temperature and other environmental factors. The particle would tend to grow by addition of other similar material and would reach a limit and divide. It would tend to encourage synthesis of the constituent parts by whatever agency they were being built. It would respond to a stimulus by orientation or motion or growth.

From this beginning up to the realm of microorganisms there is quite room enough for the existence of whole categories of living material, including ultramicroscopic nucleate organisms, simpler organisms and unorganized virus.

A virus might arise *de novo* in one of three ways, by attack of a pathogen on a living cell element, by incidence of a chemical material upon the element, or by stress of marginal conditions for life.

Viruses or very simple organisms might undergo organic change, such as attenuation.

Aeroplane Dusting With Sulphur to Combat Stem Rust of Wheat.—W. W. MACKIE.

In 1935 an epiphytotic of stem rust in the San Joaquin Valley, California, was combatted by aeroplane dusting with sulphur. Approximately 25 pounds per acre were applied. All of the sulphur used passed through a 325-mesh screen and was included in 4 brands. From April 29 to May 14, 22,000 acres were dusted. The dusting operations began at daylight and continued until about 9 a. m., when convection currents of warm surface air caused the sulphur to rise in clouds. As many as 13 planes were in the field at the same time. The dust was most effectively applied between anthesis

and hard-dough stages, a period of approximately 21 days. Although 3 dustings were recommended, most of the fields received but one. One application halted the rust attacks about 7 days, when rust attacks began again and continued until checked by unfavorable weather or by the maturity of the wheat. Where the second dusting was applied 10 days after the first, very satisfactory control was secured. Uredia forming just before the second application were destroyed or halted before sporulation occurred.

An Unidentified Species of Sphaeropsis on Maize in California.—W. W. MACKIE, HELEN JOHANN, AND N. E. STEVENS

In the autumn of 1933 diseased stalks of maize were found in the plots at Berkeley. During the following year the disease appeared to increase considerably. A few isolated specimens were found in corn fields of the delta region, near Stockton. The diseased plants matured somewhat prematurely, bearing stunted ears with shriveled kernels. Numerous lesions were borne on the first, second, or third internodes only, and are in appearance much like uredia of stem rust. These lesions erupt while the ears are maturing. The prematurely ripened stalks become brittle and readily break under wind or other stresses. Helen Johann, of the Division of Cereal Crops and Diseases, United States Department of Agriculture, placed the fungus in the genus Sphaeropsis. On media consisting of agar and cornstalk shavings the mycelial growth was light, but pycnidia were produced bearing unicellular pycniospores. On oat agar a heavy grayish green stroma developed but no pycnidia appeared. The spores probably averaged 15 μ in length, which is short for the common *Sphaeropsis malorum* but is not greatly different from *S. ambigua* (Saccardo 3: 304), the lone species so far discovered reported on corn. An examination of old cornstalks that had over-wintered at Berkeley disclosed only insect-mutilated pycnidia from which the perfect form could not be identified. Further identification will be necessary before this species of Sphaeropsis can be definitely placed.

Observations on a Species of Ligniera, a Root Parasite of Stellaria media.—J. T. BARRETT AND HORACE PARKER

While making a survey of chytridiaceous parasites of root and root hairs of higher plants a species of Ligniera was found abundantly and in all stages of development in *Stellaria media.* A comparison with the published descriptions of *L. junci* (Schwartz) Cook suggests that our form is that species. A careful study of living material suggests that the life cycle starts by infection of the plant by swarm cells originating from resting spores. The resulting myxamoeba give rise to zoosporangia that produce a few (1–6) biciliate zoospores. These develop rapidly, retaining a more or less spherical shape at first, and give rise to the groups of resting spores. The cilia, one long and one short, are retained on the growing zoospore within the host cell often until the zoospore has quadrupled in size. Germination of the zoosporangia and penetration of the host wall have been observed but not the germination of the resting spores. Empty spore cases have frequently been seen in partially disintegrated roots.

A Phytophthora Root Rot of Cauliflower.—C. M. TOMPKINS, C. M. TUCKER, AND M. W. GARDNER

A root rot of cauliflower, *Brassica oleracea* L. var. *botrytis* L., caused by *Phytophthora megasperma* Drechsler, occurs in winter plantings in the coastal areas of central California. Low, poorly drained areas and low temperatures are the principal predisposing factors. Characteristic symptoms consist of reddening or purpling of the leaves, sudden wilting, and invasion and decay of the root system. Plants of all ages are susceptible. Cauliflower seedlings (6 to 8 leaves), in 6-inch pots of autoclaved soil in

buckets containing 4 inches of water were inoculated with pure cultures of the fungus, and placed outdoors at 14° to 20° C. The incubation period is approximately 3 weeks. Healthy cauliflower seedlings were subsequently inoculated and infected with the reisolated fungus. The growth optimum of the fungus on malt extract agar is 19° to 22° C. Brussels sprouts, *B. oleracea* var. *gemmifera* Zenker; cabbage, *B. oleracea* L. var. *capitata* L.; and stock, *Mathiola incana* R. Br., are subject to natural infection, while stock and wallflower, *Cheiranthus cheiri* L., were artificially infected. The fungus produces oogonia and oospores resembling those of *P. megasperma*, but slightly smaller; both amphigynous and paragynous antheridia are present, the latter in much greater numbers. Sporangia, which are rarely found, are nonpapillate. In cultural characters and temperature relations the fungus behaves identically with *P. megasperma*. The differences in sizes of oogonia and oospores are regarded as too small to warrant the separation of the cauliflower fungus, especially in view of the very close correspondence in other characters.

Crown Gall on Conifers.—C. O. SMITH

Conifers have only recently been listed as attacked by the crown gall organisms, *Pseudomonas tumefaciens* Smith and Townsend. The literature records natural crown galls on *Cupressus arizonica* Green and *Juniperus sabina* Linn. W. T. Horne, Plant Pathologist, University of California, in conversation with the author, reported observing in 1910 a lawn tree of *Libocedrus decurrens* that had a typical crown gall 3 to 4 inches in diameter. In 1916 the author studied a gall on nursery trees of *Libocedrus decurrens*, isolated the causal organism, *Ps. tumefaciens*, and produced, with these cultures, galls on *Salix* sp., on *Prunus cerasifera*, on *Cupressus sempervirens*, and on tomato. Galls from puncture inoculation with *Pseudomonas tumefaciens* isolated from peach have been produced during 1934 and 1935 on the following species of conifers: *Cupressus arizonica*, *C. arizonica* var. *bonita*, *C. lusitanica*, *C. guadalupensis*, *C. macrocarpa*, *C. sempervirens*, *Chamaecyparis lawsoniana*, *Juniperus procera*, *J. cedrus*, *J. hierbernica*, *Thuja orientalis*, *T. plicata*, *T. occidentalis*, *Sequoia sempervirens*, *S. gigantea*, *Pseudotsuga taxifolia*, and *Libocedrus decurrens*. Crown gall is produced artificially on conifers with great difficulty. The inoculation wounds heal over with some swelling of tissue around the injury, and the results at first appear to be doubtful or negative, but some months later several knob-like enlargements may develop from the healed tissue. These are small (1 to 2 millimeters), but they continue to increase in size and may coalesce. On *Sequoia gigantea* galls more typical in size and shape have developed.

An Outbreak of Curly Top on Pansy.—B. F. DANA AND F. P. MCWHORTER

An important pansy-seed industry in Oregon was menaced in 1934 by a serious outbreak of curly top. On July 6, 20 per cent of the plants in a 5-acre field showed severe injury. Mild injury appeared on many other plants. No difference in susceptibility could be detected in named varieties or variety mixtures, excepting a blue-flower foreign strain with 80 per cent severely injured plants. Dwarfing and rosetting of shoots were prominent symptoms. Flower size and seed production were strikingly reduced. Diseased plants ceased blooming early and gradually deteriorated, many dying before the end of the season. Adults and nymphs of the beet leaf hopper, *Eutettix tenellus*, were abundant on all plants examined July 6. The severely diseased plants and the large numbers of nymphs indicated that the pansy was a favorable host for the leaf hopper and also for the curly-top virus. Plants grown from seed produced by diseased plants were disease-free, thus indicating lack of seed transmission of the curly-top virus. This also was in line with experience of the grower in perpetuating breeding stock.

Root rot of aster caused by Phytophthora cryptogea.—C. M. TOMPKINS, C. M. TUCKER, AND A. E. CLARKE

Root and stem rots caused by *Phytophthora cryptogea* have been reported on *Aster* sp. in England and Ireland and on China aster, *Callistephus chinensis*, in Australia. In August, 1934, a root disease of China aster, caused by *P. crytogea*, was observed in Golden Gate Park, San Francisco, and later in aster plantings in other coastal areas. Low, poorly drained soils favor infection. Fungus invasion occurs at or near the base of the tap-root and progresses upward to the base of the petioles, producing a blackish brown discoloration of the tissues. Root invasion causes a sudden loss of turgidity in the leaves with complete collapse of aerial parts, followed by early death of the plant. Young, healthy China asters, variety Late Branching Mary Semple, grown in 6-inch pots of moist, autoclaved soil, were successfully inoculated in the greenhouse at 18° C. with pure cultures of *P. cryptogea*. Aster plants of the same variety were later inoculated with the reisolated fungus and infection obtained. In greenhouse tests complete susceptibility to infection was shown by 45 varieties of China aster in 10 classes, including Fusarium wilt-resistant types. On oatmeal agar the fungus produces abundant white cottony mycelium with occasional clusters of small, hyaline, thin-wall vesicles, but no sporangia or oogonia. Hyphae transferred to Petri's solution or a nonsterile soil suspension develop long-ovate to obpyriform, nonpapillate sporangia averaging about 33 x 17.5 microns, with a small, refringent, thickened region at the apex. Ooogonia were not observed. Optimum growth occurs at 25° to 28° C. and ceases at 35° C. In morphologic, physiologic and pathogenic characters the fungus agrees closely with *P. cryptogea*. This is believed to be the first definite record of the occurrence of the species in America.

Observations on the Pathological Histology and Phyto-chemistry of Psyllid Yellows.—J. R. EYER.

The manner of entrance of the feeding stylets of the potato psyllid nymph into the vascular tissues of the leaf is described. The destruction of border parenchyma cells at the feeding objective and the deposits of starch in the surrounding mesophyll of the leaf and the cortex and pith of the stem are characteristic of the disease. No x-bodies were observed in diseased foliage, nor was any necrosis of the phloem of the stem apparent. The chloroplasts revealed disintegration, especially near the feeding punctures and generally throughout leaf tissues showing marked disease symptoms. The sheath materials surrounding the stylets are apparently of animal origin, and contain chitosan and musin. Certain plant tissue disintegration products also were demonstrated in the surrounding tissues. These contained arginine, tryptophane, and possibly tyrosine. Quantitative estimations of the carbohydrates in healthy and diseased plants confirmed the abnormally high starch values for diseased leaves and stems, although it was further observed that these decline rapidly after the plant reaches maturity. The definite positive relationship between hexoses and starch present in the healthy plant is abrogated by the disease. Sucrose, the principal sugar of translocation, was found to be absent or its formation inhibited in the vegetative portions of diseased plants.

Toxicity of Compounds of Ammonia to Sclerotium rolfsii.—A. E. DAVEY AND L. D. LEACH

In field trials, reported elsewhere, it was found that in soils of pH 7.0 and pH 7.9 ammonium sulphate was as effective as anhydrous ammonia in reducing loss to sugar beets from southern sclerotium rot. In an attempt to explain the action of ammonium sulphate, laboratory tests were made of the effect of ammonium sulphate in solutions buffered with calcium carbonate, solutions of ammonium carbonate (bicarbonate) and ammonium phosphates.

It was found that solutions of ammonium sulphate containing 82.4 millimols of

ammonia (NH_3) per liter (pH 6.0) and solutions of monammonium phosphate with the same ammonia content (pH 4.5) did not injure mycelium of *Sclerotium rolfsii* immersed in them. Solutions containing 41.2 millimols of ammonia as ammonium sulphate and saturated with calcium carbonate giving a pH of 7.8 were toxic to mycelium within 5 days. Solutions of ammonium bicarbonate and dibasic ammonium phosphate with a pH of 7.5 and concentrations respectively of 17.8 and 14.7 millimols of ammonia were also lethal, within 5 days, to immersed mycelium.

The above results suggest that ammonia, applied to the soil as solutions of anhydrous ammonia or ammonium salts, may, with a sufficiently high pH, be the effective agent in reducing the extent of infection by inhibiting the growth of the fungus.

Soil Amendments for Southern Sclerotium Rot of Sugar Beets.—L. D. LEACH AND A. E. DAVEY

The percentage of infection of *Sclerotium rolfsii* on sugar beets was reduced from 60 per cent in untreated plots to 25 per cent in plots treated with cyanamid at the rate of 1000 pounds per acre applied 3 weeks prior to planting. In the same experiment, plots treated with 10 tons of manure per acre showed 40 per cent infection. Hydrated lime at 5 tons per acre or ammonium sulphate, side-dressed at 1000 pounds per acre, did not significantly reduce the percentage of infection. The yields in tons per acre of disease-free sugar beets from the plots were as follows: Untreated, 7.8; cyanamid, 16.8; manure, 11.1; hydrated lime, 10.9 and ammonium sulphate, 10.8.

In another series of plots, application of either anhydrous ammonia of ammonium sulphate in irrigation water at the rate of 200 pounds of nitrogen per acre decreased the percentage of infected beets from 30 to 9 per cent and increased the yield of disease-free beets from 11.5 to 19.7 tons per acre.

Applications of anhydrous ammonia in irrigation water to sugar beets, after 6 per cent of the plants were killed by the fungus, reduced the final percentage of infection but did not provide a practical control.

The Relation of Penicillium vermoeseni to a Disease of Ornamental Palms.—DONALD E. BLISS

A leaf-base rot of *Phoenix canariensis*, a bud rot of *Washingtonia filifera*, and a trunk canker of *Cocos plumosa* are responsible for the loss of many ornamental palms each year along the Pacific Coast in southern California. Although differing in outward manifestations, these diseases are similar as to cause. *Penicillium vermoeseni* Biourge (*P. roseum* group) was isolated from the affected tissues of the 3 palm species. Controlled inoculation and reisolation experiments with these isolates, together with a similar culture from *Phœnix dactylifera*, proved the pathogenicity of each isolate on its respective host. Cross inoculations using cultures from *Ph. canariensis* on *W. filifera* and *C. plumosa* indicated that the various cultures of *P. vermoeseni* were alike pathogenically and about equally virulent. Inoculations were made through wounds, except on *W. filifera* in which moldy bran was shaken between young, folded pinnae.

Dissection of diseased palms showed that in *Phoenix canariensis* death was caused by the successive decay of leaf bases from the oldest to the youngest; that in *W. filifera* it was due to mycelial invasion of the young, tightly folded leaves in the bud; and that in *C. plumosa* it was caused by the weakening and breaking of the trunk.

The Symptoms of Narcissus Mosaic Developed Within the Plant.—FRANK P. MCWHORTER

Narcissus mosaic leaf symptoms, evident externally, result from demonstrable cell and tissue changes within the epidermal and outer chlorenchymatous regions. The squarish mottle or mosaic pattern is in part delineated by the cross-veining that forms

a right-angle network within the narcissus leaf. Roughening of the leaf and stem surface is a true and important symptom of narcissus mosaic. In all positive mosaic cases investigated cytologically, hypertrophy of epidermal cells or of chlorenchymatous cells just beneath the epidermis, is a constant symptom of diagnostic value. X-body formation occurs in some varieties of mosaic-diseased narcissus but is of little diagnostic value. Initial hyperplastic activity within chlorenchyma subtended beneath the affected epidermis produces epidermal galls. The cells composing these exhibit coarse vacuolation and are bounded by walls broken down into component striae. Cuticular membranes are disrupted. These cell changes exhibit facilities for excess water loss and partly explain the tendency to wilt characteristic of mosaic individuals.

A Peach Tree Disease Recently Discovered in California.—H. S. REED AND H. H. THORNBERRY

The disease came into prominence in one of the important peach districts of southern California in the spring of 1933 and has increased in severity and distribution each succeeding year. Since the nature of the disease has not been determined, we have temporarily designated it as the "1933 peach tree disease." Trees affected by it are not killed but assume an extremely unthrifty condition. Fruit production is reduced to a point where the orchards do not pay expenses. The leaves of affected portions of trees are small and usually show yellowing or irregular areas that occasionally drop out, forming numerous holes. Severely affected leaves fall from the trees by the end of June and are succeeded by apparently healthy ones. The first foliage, however, produced the following spring, again shows typical symptoms, and the tree is less thrifty than in the preceding year. Similar symptoms have been found on almond, plum, and prune foliage. Experiments have been started to determine the transmissibility of the disease, and observations are in progress to learn something about the susceptibility of different varieties.

Endoxerosis of Lemon Fruits as Affected by the Application of Different Amounts of Irrigation Water.—E. T. BARTHOLOMEW

Lemon trees were grown in large lysimeter tanks. The soil had a moisture equivalent of 11 per cent and a wilting point of 4 per cent. Water was applied to one group of these trees when the soil had reached a moisture content of 6 to 8 per cent; to the second group when it had reached 5 to 6 per cent; and to the third group when it had reached 3½ to 5 per cent. The yields of endoxerotic fruits, arranged in descending order, were third, first, and second groups, respectively. On the whole, the results of these tests indicate that, while a high soil moisture content is conducive to the production of endoxerotic fruits, yet, when the period of stress (hot weather) is great and is sufficiently extended, those trees having access to the least amount of water will show the greatest yield of endoxerotic fruit.

The Interpretation of Oregon Tip Blight on a Basis of Causal Viruses.—FRANK P. MCWHORTER AND J. A. MILBRATH

During the last 4 years (1930–1935) a tomato disease, locally called tip blight, has been of great economic importance in the Southern Oregon canning areas. Typical diseased plants exhibit severe blighting of the terminal shoot as the most noticeable symptom. Small plants are killed; larger (older) plants soon lose their ability to produce marketable fruit. The general condition suggests "streak" but this interpretation has been rendered untenable both by symptom study and by test inoculations. Inoculations by grafting and by carborundum methods relate the Oregon trouble to

two viruses: "Spotted Wilt" and an undetermined virus. The undetermined one produced (1934) most of the tip-blight cases, is very difficult to transfer from tomato to tomato, and has thus far failed to produce symptoms on any other solanaceous host. On tomato it is a quick-killing virus, selectively active on terminal shoots, and first evidenced by arrested growth and black, usually large, necrotic spots on leaves.

The Properties and Interpretation of Tulip-Breaking Viruses.—FRANK P. MCWHORTER.

The theory that tulip breaking results from the interaction of two antithetic viruses one of which tends to add flower color and one to remove flower color, has been substantiated by actual synthesis of normal breaks by inoculating unbroken tulips with mixtures of viruses. The color-removing virus is dominant. A juice inoculum consisting of 10 parts of color added to 1 part of color-removing, resulted in the synthesis within a group of inoculates of a large proportion of normal breaks comparable to commercial Rembrandts.

We have been unable to distinguish or separate the 2 viruses on a physical-property basis. Some of the properties are: Thermal death point lies between 65° and 70° C.; infectious in dilutions up to 1/100,000; are not destroyed by drying (leaves) for 11 days; resistant to alcohol; easily transmitted by hypodermic injections; not transmitted by gentle rubbing; inoculations to onion, tomato, iris, narcissus, petunia, Tropaeolum, Brodea and Camassia were negative. The tulip viruses are very closely related to those frequently present within the bulb-perpetuated lilies.

An Explanation of Rhiomyxa hypogaea Borzi.—J. T. BARRETT

While making observations on a species of Ligniera in the roots of *Stellaria media*, oospores of a fungus were found that closely resembled those illustrated and described by Borzi as a part of the life cycle of his *Rhizomyxa hypogaea*. The oospores often are associated with the Ligniera, but may occur in roots in the absence of the latter fungus. Associated with this sexual stage, which was observed in all stages of development, are large oval to long zoosporangia that discharge biciliate swarm spores through very thick exit tubes. No vesicle has been definitely observed. As seen in roots of *Stellaria media*, the zoosporangia occur singly. Very recently the same sexual stage was observed in the roots of sugar beet associated with similar zoosporangia joined in a chain of 3 to 6. In one case all zoosporangia discharged zoospores almost simultaneously. The studies thus far made suggest that Borzi's *Rhizomyxa hypogaea*, as described, may have been an association of Ligniera, perhaps *L. junci* (Schwartz) Cook, and an undescribed species of the order Ancylistales.